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EFFECT OF STORAGE TEMPERATURE, DURATION AND DRYING TECHNIQUE ON THE MAJOR VOLATILE ORGANIC COMPOUNDS IN MANGO GINGER (*Curcuma amada* Roxb.)

Thesis submitted for the degree of Professional Doctorate in Sustainable

Food Quality for Health (DAgriFood)

Department of Food and Nutritional Sciences

By

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December 2021

Declaration

I confirm that the work presented in this thesis is my own and that the use of all the literature from other sources has been properly and fully acknowledged.

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December 2021

Abstract

Curcuma amada Roxb. is an important member of the Zingiberaceae family and is commonly known as mango ginger due to its resemblance to ginger and the green mango like aroma of the rhizome. The biochemical profile of mango ginger is characterised by a number of volatiles that have application in the food and pharmaceutical industries. However, due to the niche nature of where mango ginger is cultivated, commercial production and extraction of these volatiles have not been the subject of research. There is also insufficient literature on the effect of post-harvest processing techniques on the sensory attributes of mango ginger. As a result, harvested mango ginger rhizomes are often transported and stored alongside other major agricultural produce, without a clear understanding of the optimum storage temperature and duration required for the rhizomes. This study focuses on the changes in the content and composition of volatile organic compounds (VOCs) in mango ginger when subjected to different storage temperatures and durations. Additionally, the study also explores the effect of two commercially followed drying techniques on the biochemical profile. SPME-GCMS analysis of the change in chemical profile was also complemented by sensory analysis as part of this study.

The first phase of this study was aimed at improving the current practice prevalent among wholesalers and retailers, of storing mango ginger rhizomes for a short period without undergoing any commercial post-harvest treatments. The intention of the second phase was to determine whether commercial post-harvest treatments such as drying followed by long term storage were feasible options for mango ginger, with a view to explore new avenues on commercial use of the dried powder. This is the first report that analyses the combined effects of drying techniques and storage duration on the organoleptic properties of mango ginger rhizomes. To this purpose, this study explored the effect of three storage temperatures (8 °C, 15 °C and 22 °C) across three storage durations (1, 15 and 29 days) during its first phase. The analysis was extended to its second phase by utilising two drying techniques (hot air drying and freeze drying) and storing the rhizomes for three different storage durations (1, 2 and 3 months).

The study concluded that monoterpenes make up a significant proportion of the VOCs in mango ginger, which provide the signature aroma and flavour characteristics. In terms of relative abundance, the major terpenes were identified as myrcene, β-(E)-ocimene, β-pinene, eucalyptol, epoxy myrcene and α-pinene. Other compounds like alcohols, aldehydes, ketones, and esters make up the rest of the VOC composition and are responsible for imparting fresh, floral, and spicy notes. SPME-GCMS and sensory analysis in phase 1 confirmed that storage duration had a more significant impact on the VOC profile than storage temperature. The highest relative abundance of terpenes was identified in samples stored at 15 °C for 15 days. Principal Component Analysis (PCA) also aligned with these findings by displaying a strong positive correlation between the flavour and aroma characteristics of green mango and mild turmeric for these samples. Phase 2 concluded that the combined effect of both drying technique and storage duration was able to significantly impact the biochemical profile. Significant variations in relative abundance values were seen in different chemical groups, along with the formation of new compounds like alkanes and alkenes, and the total absence of alcohols, when compared to that of fresh rhizomes. These trends can be attributed to the formation of secondary metabolites produced by the plant in response to stress induced by mechanical injury during peeling and slicing, followed by Maillard and Strecker reactions that occur when the samples were exposed to drying. Sensory analysis in phase 2 confirmed that samples that were hot air dried and then stored for up to two months were able to retain most of the aroma and flavour attributes of fresh rhizomes. PCA also aligned with these findings by displaying a strong correlation between green mango and mild turmeric aroma and flavour compounds in these samples.

This study opens up other avenues of research, such as the use of gas chromatography – olfactory analysis, to determine the aroma of individual chemical groups and identify the most dominant odour active compounds present in mango ginger. Other methods like vacuum drying need to be explored to optimise conditions such as temperature, drying time and pressure to achieve an end product with desirable organoleptic properties and shelf-life for commercial use of the essential oils in the food and pharmaceutical industry. The results could then be combined with sensory

and consumer trials to determine the acceptance of the flavour and aroma attributes of mango ginger by the end user.

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ACKNOWLEDGEMENTS

Firstly, I would like to express my immense gratitude to God for helping me realise one of my biggest dreams in life and overcoming all the challenges to complete this thesis. I want to precede my thankfulness to my supervisor Professor Carol Wagstaff for her guidance, arranging funding for this project, constructive criticism and her friendship in the preparation of this thesis. I am particularly grateful to my husband Sanjay, my two lovely daughters Arunima and Aarya, our 'lockdown puppy' Picasso, my parents, Mr. Ayyappan K.S. and Mrs. Ramalekshmi Radha, my sister Dr. Sindhukala, my nephews Sidharth and Aditya, and my father-in-law Mr. Gopalan Purushothaman.

I would like to thank to all the members of staff, colleagues, and personnel of the department of Food and Nutritional Sciences at the University of Reading, in particularly Dr. Bola Oloyede, Dr. Stella Lignou, Dr. Lucy Turner and Mrs. Sue Mouring, Mr. Rahul Kumar, Dr. Martin Chadwick, Miss Ana Contente and Mrs. Manik Puranik for their advice and assistance with sensory trials, statistical analysis and handling complex datasets. I extend my thanks to Fiona Lee and Cathy Crips at the AFTP for all their help with admin support, Dr. Steve Elmore for guidance provided during the GCMS analysis, Dr. Sameer Khalil Ghawi and Mr. Chris Bussey for helping me in the pilot plant with processing equipment and samples storage equipment which form the foundations of this work. It would also be remiss of me if I didn't thank Mr. Ashok Chowdary, owner of the Fruity Fresh company for his courteous and professional approach in ensuring a prompt supply of mango ginger samples all the way from India at relatively short notice, despite the COVID-19 induced restrictions.

I would also like to offer my thanks to Ms. Sue Bonard in the student welfare team, and all of my friends including Ann Alex, Susan Eapen, Eapen Varghese, Richard and Xarifa, Mr. Mohan Rao Kongara, Santini, Sreejith, Anita Sakthi and Yamuna Milan, who helped and supported me throughout this study.

ABBREVIATIONS AND SYMBOLS

AEDA	Aroma extract dilution analysis
cfu	Colony forming unit
D0	Day 0 – Date on which the samples arrived at the University of Reading
D1	Day 1 – The day following day 0
D15	Day 15 of shelf-life
D29	Day 29 of shelf-life
FD	Freeze Drying
GC-O	Gas chromatography-olfactometry
GC(FID)	Gas chromatography flame ionization detector
GCMS	Gas chromatography–mass spectrometry
HAD	Hot air drying
HSSPME	Headspace Solid Phase Microextraction method
LCMS	Liquid chromatography–mass spectrometry
M1	Month 1 of the storage time
M2	Month 2 of the storage time
M3	Month 3of the storage time
MEP	Methyl erythritol
MVA	Mevalonic acid
NIST	National Institute of Standards and Technology
T1	Temperature at 8 °C
T2	Temperature at 15 °C
T3	Temperature at 22 °C (control)
VOC	Volatile organic compounds

CHAPTER 1

1. Literature Review – Discussing influencers of the total volatile content in mango ginger (*Curcuma amada*) from farm to fork

1.1 Introduction

The genus *Curcuma*, within the family Zingiberaceae, comprises about 100 accepted species of perennial rhizomatous herbs that originated and are distributed across the tropical and subtropical regions of Asia, Australia, and South America (Velayudhan, 1996; Apavatjrut et al., 1999; Islam, 2004; Sasikumar, 2005; Ravindran et al., 2007). A number of species from genus *Curcuma* are typically grown on a large scale in India, Pakistan, Indonesia, Malaysia, Nepal and Indonesia (Chuakul and Boonpleng, 2003) whilst also extensively cultivated in Bengal, China, Taiwan, Sri Lanka, Peru, Australia, and the West Indies (Ravindran et al., 2007; Policegoudra et al., 2011). Curcuma species are greatly valued for their medicinal properties and for hundreds of years, members of this genus like turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) have been used in folk and traditional medicine for treating respiratory complaints, joint pain, digestive disorders, inflammatory conditions, wounds, hypercholesterolemia, hypertension, hematologic and circulation abnormalities, infectious diseases, and cancer (Shahrajabian et al., 2019, Grzanna et al., 2005). Other species belonging to this genus, such as cardamom (*Elettaria cardamomum*) and galangal (*Alpinia galanga*) are important sources of flavouring used in cooking, colouring agents, cosmetics, perfumes, dyes and also used as ornamental plants (Velayudhan, 1996; Islam, 2004; Basak et al., 2010; Policegoudra et al., 2010; Leong-Skornikova and Newman, 2015; Akarchariya et al., 2017; Sun et al., 2017; Dosoky et al., 2019).

Historical evidence suggests that the genus *Curcuma* was first established by Carl Linnaeus in 1753. The name Curcuma is derived from the Arabic word ‘Kurkum’ meaning “yellow,” which refers to the colour of the rhizome (Syamkumar and Sasikumar, 2007). Extensive research has been undertaken into the properties and benefits of two important members of Zingiberaceae, namely turmeric and ginger which significantly popularised their usage commercially. However

other important species such as *Curcuma zedoria* and *Curcuma amada* appear to be unexplored and unexploited (Policegoudra et al., 2011; Saipriya et al., 2017; Shirsath et al., 2017) as these species are not cultivated on a commercial scale. *Curcuma amada* is an important member of this genus and closely related to *Curcuma longa*, more commonly known as mango ginger. Mango ginger is believed to have originated in the Indo-Malayan region and is widely distributed in the tropical regions of Asia, Africa and Australia (Sasikumar, 2005). Most of the documented information relating to the traditional uses of mango ginger is sparse; with some literature only being available in local languages and others are difficult to access (Jatoi et al., 2007). Accessible research identified mango ginger to possess numerous valuable properties including as an antioxidant, and having antibacterial, antifungal, or anti-cancerous attributes as a consequence of its phytochemicals. These compounds have been isolated and characterised by various methods and have a good bioavailability and possess these health benefits (George et al., 2015).

Mango ginger shares similar attributes to ginger, from the appearance of peelable skin that covers the yellow rhizome to the distinctive trigeminal effects that is commonly experienced upon consuming raw mangoes (Vishnupriya et al., 2012). Furthermore, ginger is regularly used in cooking due to its distinctive taste and flavour profile, fundamental in Asian cooking to add spice, heat and fragrance to many dishes. Both mango ginger and ginger share a similar flavour profile, comprising of a mixture of monoterpenes, sesquiterpenes, alcohols and aldehydes (Ravindran et al., 2012). Multiple studies have been completed investigating the aroma profile of ginger, most commonly using gas chromatography mass spectrometry (GCMS) to separate and identify the volatile compounds that contribute to the distinct ginger flavour. Compounds including myrcene, caryophyllene and α-thujene have been identified with odour descriptors including woody, earthy and peppery (Sharifi-Rad et al., 2017). With such a strong focus on ginger flavour, little attention has been given into the investigation of the aroma profile of mango ginger along with the influence of supply chain processes upon the composition and how these may alter sensory perception.

1.2 Mango ginger – morphological traits and distribution

Mango ginger is a perennial, rhizomatous and aromatic herb from the Zingiberaceae family. It has been used as a spice in India since time immemorial (Mustafa et al., 2005) and is found in the wild as well as cultivated in certain locations in India (Jatoi et al., 2007). The rhizome morphologically resembles that of ginger; however, when they are freshly cut, they impart the aroma, flavour, and colour of raw mango (Mangifera indica (Sasikumar, 2005; Policegoudra, 2007; Policegoudra et al., 2011; Ravindran et al., 2012).The rhizome also does not have the same sharp, pungent taste of ginger, and is milder on the tongue (Rao et al., 1989).

Mango ginger is also reported to be botanically and morphologically related to turmeric (*Curcuma longa*) (Amit Baran Sharangi, 2018). Mango ginger is also known as Amada or Amahaldi (Padalia et al., 2013), Manga Manjal, Amra Harida, Taldiha, Sarabasa, Amargandhi, Talia, Sarabanghati and Banhaldi in different regions within India (Sajitha et al., 2014; Gupta et al., 2015). The rhizomes are called "Amada" in Bengali because of the characteristic odour of mangoes, superimposed over the mild turmeric and ginger odour (Gholap and Bandyopadhyay, 1984; Rao et al., 1989). Mango ginger is known by different names in other parts of the world depending on its location. It is called Ama Adrak in Pakistan, Temu Mangga in France, Mangoingwer in Germany and mango ginger in the UK (Al-Qudah, 2017).

In Malaysia, it is also known as *Curcuma mangga*/ temu pauh/ kunyit mangga, meaning mango-like turmeric (Malek et al., 2011). The aroma profile of Malaysian *Curcuma mangga* Val. & van Zijp. rhizomes was investigated by (Wong et al., 1999), it was reported to possess a mango-like aroma and is considered to be synonymous with *Curcuma amada* Roxb., which is cultivated in north-eastern India.

1.2.1 Habitat

Although mango ginger is grown in various climatic, topographic and soil conditions it prefers a hot climate with high rainfall and exhibits maximum growth and yield under humid, tropical conditions. For this reason, southeast Asia and Southern continents are the largest producers of mango ginger in the world but there are reports of mango ginger found in Japan, Australia, China, Pakistan, Thailand and Malaysia (Policegoudra, 2007; Al-Qudah et al., 2017). Mango ginger is widely cultivated in India, although not on a commercial scale (Ghani, 1998) yet the geographical distribution and cultivation is spread across Kerala, Tamilnadu, Karnataka, Gujarat, Uttar Pradesh, West Bengal, Konkan and in the hills of the Western Coast (Padalia et al., 2013; Mustafa et al., 2005; Tamta et al., 2016).

1.2.2 Optimal growing conditions for mango ginger

Mango ginger is cultivated in a manner similar to that of turmeric in India (Syamkumar and Sasikumar, 2007; Amit Baran Sharangi, 2018). Well-developed whole or split mother rhizomes which are healthy, disease-free and weighing 15-20 g, are used for planting where they are placed into open or semi-shaded locations with a well-drained sandy loam soil. Seeded rhizomes are planted 30 x 30 cm spacing at 4 – 5 cm depth and a good crop yields up to 20-30 tonnes of fresh rhizome per hectare (Balasubramanian et al., 2016). To ensure optimal growth, the soil is well prepared to a fine tilth during February and March, with planting taking place during April to coincide with the commencement of pre monsoon showers. A single basal dose of well decomposed farmyard manure (20-30 tonnes / ha) and split doses of major nutrients NPK (30:30:60) is given (half dose of K as basal, half dose of N 30 days after planting and the remaining dose of N and K 60 days after planting). Mulching with green leaves or paddy straw is provided after planting and it is repeated after every 50 days (Balasubramanian et al., 2016).

The plant grows well in fertile soils with good drainage and is usually grown as a main crop under open conditions and it is often grown in vegetable gardens as a rotational crop with Brinjal (*Solanum melongena*) and Okra (*Hibiscus esculentus*) (Mustafa et al., 2005). A study conducted

(Jayachandran and Nair, 1998) whereby mango ginger was grown under varying shade levels revealed that mango ginger expressed excellent shade tolerances and concluded that it was therefore suitable for intercropping in coconut gardens and short duration vegetable crops. When compared to related species like ginger and turmeric, there are fewer incidences of pests and diseases for mango ginger however, the attack of shoot borer (*Conogethes punctiferalis*) has been found to cause damage to the crop when cultivated on a large scale in monocropping systems (Amit Baran Sharangi, 2018).

1.2.3 Morphology and anatomy

The plant has an erect to semi-erect stature and grows to 60–90 cm in height and the crop duration is 6 – 8 months (Apavatjrut et al., 1999; Sasikumar, 2005; Jatoi et al., 2007). Each plant bears five to six pairs of leaves which are long, petiolate, oblong, lanceolate and tapering at both the ends. They are green on both the sides but are glabrous on the upper side and puberulous on the lower side. The inflorescence is lateral or central which grows out on a separate stem from the leaf stem. It has a long and erect peduncle covered with 5-6 sheaths and hidden by the sheathing bases of the leaves. The inflorescence has pale green or straw-coloured fertile bracts. The flowers are long and large with 4-5 flowers in each bract. These bracts have a tuft of pale purple or rose-coloured barren bracts or leaves at their tip.

1.2.4 The Rhizome

The root stock is ovoidal or conical in shape; the rhizomes are branched and large, which normally appear buff coloured. Tubers are cylindrical, thick fingered and fleshy which normally arise from the base of the rootstock whereas the rhizomes are fleshy, five to ten centimetres long and two to five centimetres in diameter, with demarcated nodes and internodes. Rhizome flesh is light to pale yellow in colour with a distinct core emitting the fragrance of unripe mango when crushed and the surface of the rhizome has growth rings with scars, resulting from the circular arrangement of scaly leaves at the nodal region. Sympodial type of branching can be observed on the rhizomes. When fully mature, each plant yields up to 2 kg of rhizome (Policegoudra, 2007) and the main

rhizome and fingers vary in weight from 20 to 75 g. They exhibit the colour, flavour and aroma of raw or unripe mangoes when crushed and are pungent in taste upon consumption with an initial bitter taste which develops into sweet tasting before becoming sour and aromatic to the palate. Typically, mango ginger has been reported to display a similar taste to its Indonesian counterpart, *Curcuma manga*, and thus, its culinary uses are similar (Khare, 2008; Ravindran et al., 2012).

The rhizomes are made up of 86 % water, 0.1 % essential oil, 0.1 % of curcumin, 0.8 % ash, 1.4 % fibre, 0.8 % total sugars and reducing sugars, and 6.9 % starch on fresh weight basis. On a dry weight basis, they have 45.6 % starch (of which 43 % is amylose), 0.9 % essential oils, 10.6 % crude fibre, 5.7 % ash, 5.8 % total sugars and traces of reducing sugars Myrcene (78.6 %), β-(E)-ocimene (5.1 %), β-pinene (3.7 %) and α-pinene (2.9 %) were identified as the major VOCs in the rhizome, using capillary GC and GCMS (Mustafa et al., 2005; Policegoudra and Aradhya, 2007; Policegoudra and Aradhya, 2008; Amit Baran Sharangi, 2018). The remaining compounds were made up of sesquiterpenes, alcohols, aldehydes, ketones and esters (Table 2 of Chapter 2).

Spices and herbs are rich sources of phytochemicals (Zheng and Wang, 2001; Shan et al., 2005; Srinivasan, 2014), often consisting of flavonoids and phenolic compounds, carotenoids, plant sterols, glucosinolates and other sulphur-containing compounds. Identified in rhizomes, tubers, fruits and vegetables, these secondary metabolites have been well studied, and shown in the literature to not only contribute health benefits to vegetables but also, to contribute taste characteristics to crops when eaten, particularly imparting bitterness and astringency (Tholl, 2006). Mango ginger is no exception, being rich in essential oils and containing over 100 phytochemical compounds, including volatile and non-volatile compounds, that have been identified to possess biomedical properties (Policegoudra, 2011). Aroma and flavour components have been isolated from different parts of the plant (Shirsath et al., 2017) and a number of phenolic, flavonoid, volatile and non-volatile components have been identified in the rhizome's essential oil (Padalia et al., 2013; Gupta et al., 2015). These bioactive compounds participate as precursors in imparting colour, aroma, flavour and defence mechanisms.

1.2.5 Harvesting

The harvesting and processing of mango ginger follows techniques similar to that of turmeric and ginger where harvesting readiness is indicated by desiccation of the leaves, this is typically six months after planting (Ernst and Durbin, 2019). Irrigation is normally stopped one month before harvest, but a final irrigation is provided two days prior to harvesting to help with uprooting the rhizomes. The initial step is to remove some senescent foliage to make the rhizome more accessible and then the soil is loosened around the crown of the plant. A spade or digging fork is used to manually uproot or lift out the whole plant carefully before the rhizomes are gently pulled out using the remaining length of the stem as a handle. In some cases, such as when a 'ridge method' of planting is used, harvesting is done using a plough (Anandaraj and Sudarshan, 2011). Very wet or very dry conditions are not recommended for harvesting as it increases the amount of wounding of the outer skin and makes the separation of rhizome from the soil more difficult (Kaushal, 2017).

1.2.6 Processing

Harvested plants are left in the field under shade for a few hours for the leaves to dry. The stem attached to the rhizome is snapped or cut off slightly above the point of attachment to the rhizome. At this point, some of the rhizomes will be pre-graded in the field, discarding those that display signs of damage or disease (Nair, 2019). Once harvested, fresh rhizomes are washed and cleaned to remove shoots, roots, and soil debris, they are trimmed and dried under shade or in open conditions out of direct sunlight for two to three hours, weather permitting, before they are sent to market or stored at ambient temperature (Kaushal, 2017). Once the rhizomes are separated from the main plant, the wounding that occurs initiates lignin formation which acts as a protective layer to the cut surface. To enable this process, the rhizomes are air dried for two to three days (Balasubramanian et al., 2016).

1.2.7 Grading and Packing

Rhizomes are packed in bulk in jute sacks, wooden boxes or lined corrugated cardboard boxes for transport to local and international markets. Temperatures for storage before and during transportation are aimed to be under ambient temperatures (this can vary from season to season as there is no temperature regulation during this process) for one to two days until they reach the point of export. The produce is then graded and sorted according to qualitative attributes such as appearance, deformity, sprouting, over / under maturity (determined by the colour of the rhizome core), undersized, and presence of pest and diseases. Graded and sorted rhizomes are packed in plastic sacks which are perforated to provide ample aeration and each bag (5 kg) is stored at 7 to 8 ° C until they are ready to be exported internationally by flight. It takes 9 to 11 hours to reach an airport in the United Kingdom, during which period the rhizomes are stored at flight temperatures between 7 and 8° C. From the airport, the produce is collected by courier lorry and transported to international licenced wholesale markets at ambient temperature for further distribution to the retailers. They are normally stored at 7 to 8° C in warehouses until distribution to retail shops. Once at the shops, they are usually displayed and stored at an ambient temperature of 20 to 22 ° C for a month, which is the maximum shelf-life before sale (Raj et al., 2016, Thomas et al., 2017).

Harvested rhizomes therefore go through a wide range of storage temperature during the supply chain (Figure 1). These drastic changes occur immediately after harvest and within a short period of a week, which can act as an environmental stress factor and subsequently cause alterations to the metabolic pathways responsible for the production and emission of Volatile Organic Compounds (VOCs) (Blanch et al., 2007). In an ideal situation, these temperature fluctuations must be maintained within tolerable limits, as cold temperatures are not conducive to extend the post-harvest shelf-life of tropical crops owing to their susceptibility to chilling injury (Kader and Yahia, 2011). However, not much research has been done to identify the optimal temperature conditions to be maintained during the supply chain cycle of mango ginger.

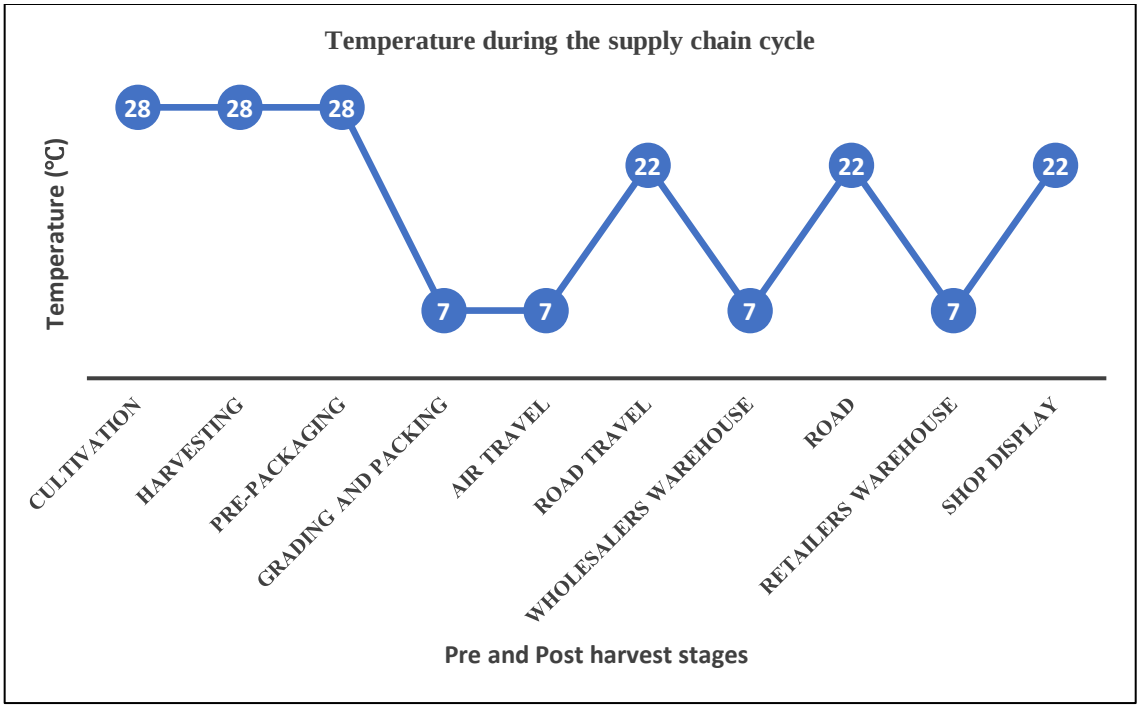


Figure 1 – Temperature regimes across the international supply chain cycle of mango ginger from cultivation to purchase by the consumer

1.2.8 Pharmaceutical uses

The presence of various phytochemicals and their beneficial effects provides great potential for mango ginger to be commercially exploited in the pharmaceutical industry. Extracts from rhizomes have been demonstrated to have antioxidant, antimicrobial, anti-cancerous and anti-inflammatory properties (Policegoudra et al., 2011) and chloroform extracts from mango ginger have shown high anti-bacterial activity against both gram positive and gram negative bacteria (Policegoudra et al., 2007a; Policegoudra et al., 2007b) which have been attributed to terpenoid compounds like difurocumenonol, amadannulen and amadaldehyde. The rhizomes are used in traditional medicine to cure skin diseases, heal wounds, promote digestion and ease bronchitis, asthma and inflammation; most of these properties have been attributed to the presence of myrcene, β-pinene, and epicurzerenone, which contributed to 6.6 %, 1.8 % and 2.5 % respectively of the essential oil. (Nayak, 2002; Sinha, 2003; Behera, 2006; Srivastava et al., 2006; Policegoudra et al., 2007b; Voravuthikunchai, 2007; Policegoudra et al., 2011). It is also used for the treatment of various intestinal diseases, jaundice, fever, sprains, bruises (Jain, 1964;

Mujumdar et al., 2000; Policegoudra et al., 2007c; Padalia et al., 2013). The protective effects against obesity related diabetes have also been reported in a number of studies (Bray et al., 2017; Rao et al., 2019; Sarkar et al., 2019). However, the commercial extraction of bio actives from mango ginger is still a gap in the pharmaceutical industry and targeted efforts are needed to investigate and quantify these phytoconstituents and the full pharmacological profile of mango ginger (Samant, 2012).

1.3 The farm to fork journey of agronomic produce

The supply chain of horticultural produce includes several activities comprising production/manufacturing, distribution and marketing, at the end of which the consumer is supplied with a desired product (Kalidas et al., 2014). Supply chain systems comprise interconnected activities (Figure 2) from the time of harvest through processing, marketing and food preparation, leading to the final decision by the consumer; whether to consume or discard the end product.

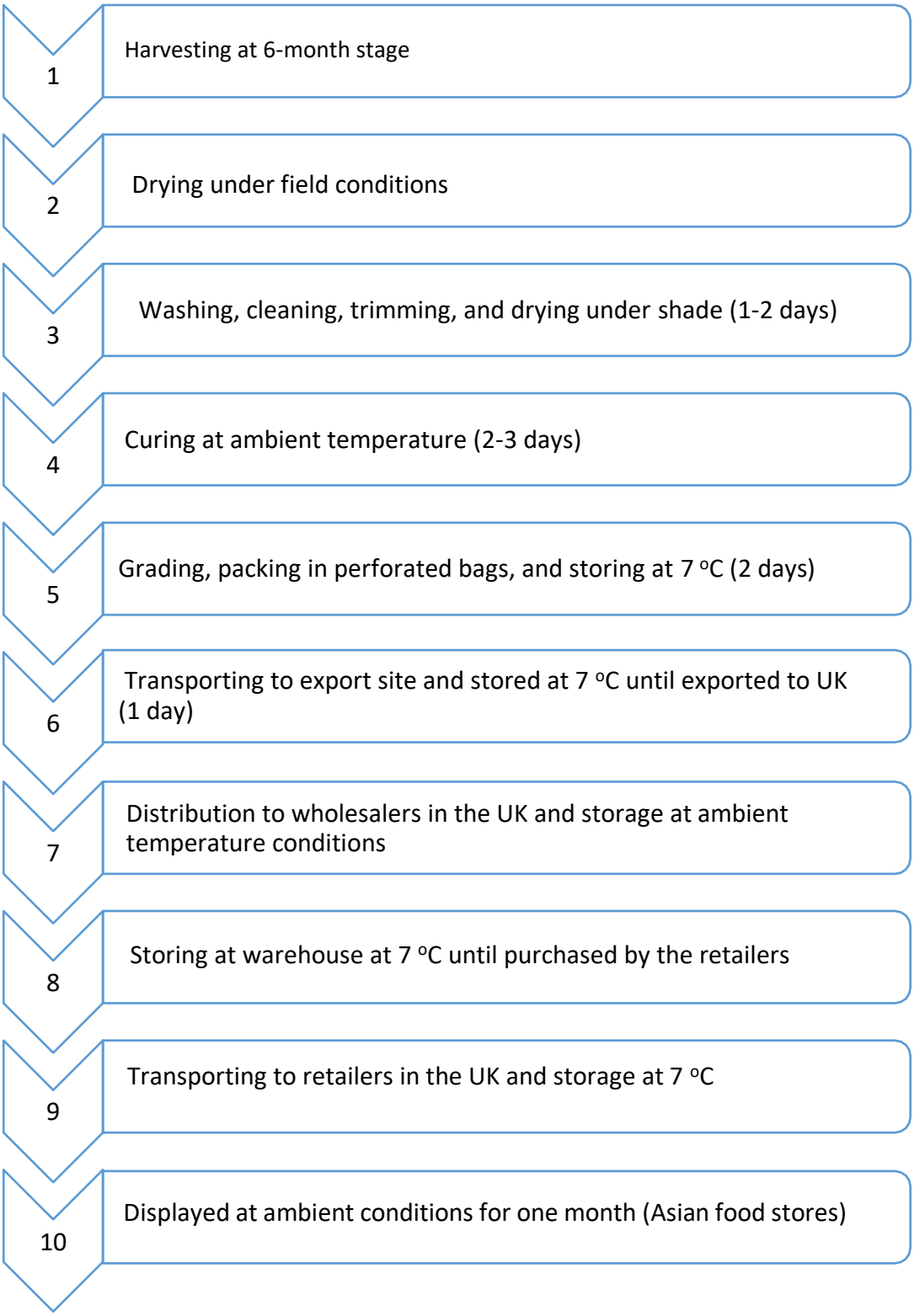


Figure 2 – Supply chain of mango ginger from India to the UK.

Losses of quantity (weight or volume) and quality (altered physical condition or chemical characteristics) can occur at any link in the post-harvest supply chain and can result in economic losses (Hodges et al., 2011). External characteristics such as appearance decides whether consumers will purchase the harvested produce, but internal characteristics such as aroma, flavour, taste, and mouthfeel will decide acceptance and repurchase. The quality of horticultural produce cannot be improved after harvest, they can only be maintained by appropriate post-harvest processing through minimising abiotic and biotic stresses and maintaining optimum storage conditions (Kader, 2013, Siddiqui, 2015; Kumar et al., 2020,). However, this is difficult to control as all produce goes through mechanical stresses when the plant-part is separated from its parent by cutting off its supply of water, nutrients, hormones, and energy. Horticultural produce remains 'alive' even after harvest, be it the root, stem, flower, or fruit and when harvested, these plant tissues have an altered ability to respond to stresses in the environment (Kader and Yahia, 2011). These stresses interrupt, restrict or accelerate normal metabolic processes in an adverse or negative manner and therefore, are usually considered as potentially injurious to any plant system (Watkins, 2017, El-Ramady et al., 2015). Post-harvest management of horticulture produce is defined as a set of post-production practices which include several steps such as cleaning, washing, selection, grading, disinfection, drying, packing and storage. These steps are mainly followed to eliminate contaminants and improve product appearance and increase shelf-life. The process also ensures that the end product complies with established quality standards for fresh and processed products for the market and finally to consumers (Santacoloma et al., 2009). Post-harvest handling is therefore considered to be the final stage in the process of producing high quality fresh produce (Bachmann and Earles, 2000).

During the supply chain life cycle, freshly harvested produce, in general is often exposed to varying surrounding temperatures from the time of harvest throughout transportation, storage, and marketing. This process is further complicated as some distributers send loads of several varieties of agricultural produce in one large container to maximise usage of available space and minimise transportation costs (Brecht et al., 2003). During retailing, the surrounding temperature

is usually higher than that during shipping or storage (Cameron et al., 1993; Brecht et al., 2003). This damage is exacerbated during the movement of fresh produce into the marketplace as some producers, wholesalers and retailers often do not have the facilities to set optimum storage temperatures for each commodity (Prussia and Shewfelt, 1993).

Maintaining a constant and optimum temperature throughout the post-harvest supply chain, from its site of production to an international retail market is considered one of the most difficult tasks to maintain quality. These limitations are especially true for exotic commodities which are handled in small quantities and consumed only by a minority of the population in the UK (Paull, 1999). Mango ginger is grown only in niche regions around the world and is not cultivated on a commercial scale. As a result, most of these regions lack proper *in-situ* post-harvest processing facilities in the form of poor cold storage facilities and it is a common practice to re-bury the rhizomes until the whole area is harvested or they are left as such in the field until they are in high demand. In the United Kingdom, the supply chain process of mango ginger lasts for a week from its centre of production until it reaches the retail supermarkets, during which the rhizome is exposed to all the above factors, putting the original harvest quality at risk.

1.4 Factors that impact the quality and quantity of VOCs

The value of a spice is significantly influenced by its unique aroma and flavour characteristics, which also determines the quality of the final product as perceived by consumers at the time of purchase. Consequently, ensuring a good quality of the harvested product across the supply chain is a prerequisite to enhance the flavour, aroma, and other nutritional components. This is important in satisfying consumer expectations and also to aid its demand through repurchasing (Hewett, 2006). Although recent years have witnessed a significant rise in the international trade of horticultural products, the need for a method of transportation which ensures a high-quality supply of these products has challenged post-harvesters across the world (Brecht et al., 2003).

Morphological qualities such as appearance, colour, size, shape, and nutritive/physiological qualities are the result of various metabolic processes taking place within the crop and these have a significant impact on the sensory and organoleptic properties of the crop including aroma, flavour and texture. Post-harvest handling and processing plays an important role in maintaining these qualities throughout the supply chain journey; it has been estimated that 15–30 % of fresh horticultural produce deteriorates to a point after which it is no longer marketable after harvest (Ali et al., 2021). Post-harvest losses recorded in tropical crops in terms of both quality and quantity are very high, especially because these fruits have to be transported across long distances from where they are cultivated to where they are finally consumed. One of the most important factors that help to maintain the shelf-life of harvested produce is storage temperature (Kader and Yahia, 2011) .

VOCs can be released as soon as they are synthesised or can be stored first in storage structures like rhizomes and then slowly emitted from them (Niinemets et al., 2010; Harrison et al., 2013). Aromatic plants which have a strong constitutive capacity for VOC storage and subsequent emission are significantly impacted by stress during storage. Stress can result in the modification of these emissions by altering the rate of synthesis of VOCs or by altering the permeability of cell walls of storage structures (Niinemets et al., 2010; Copolovici et al., 2012). Therefore, it is

important that perishable products are carefully handled at every stage to prevent deterioration of the produce between the harvest and consumption period (Dhatt and Mahajan, 2007). Ripening and senescence are the final stages in the development of tropical produce during which certain irreversible processes resulting in the breakdown of cells and ultimate death. The main aim of post-harvest handling, therefore, should be to delay activities those metabolic pathways responsible for causing senescence. Understanding the biological attributes which impact the post-harvest deterioration rate is therefore essential for extending the post-harvest shelf-life of tropical produce (Kader and Yahia, 2011).

During post-harvest transport, minor spices such as mango ginger are often bundled along with other major crops and exposed to unsuitable temperature and storage regimes. Combined with the lack of research literature on optimal post-harvest storage temperature for these niche spices, there is a significant lack of awareness among post-harvesters on how to create optimal supply chain conditions for them. Past research on herbs has focused primarily on the study of the content and composition of VOCs with few references to the changes that appear during storage (Masanetz and Grosch, 1998; Parthasarathy et al., 2008; Vokk et al., 2011). Although herbs (basil, lemongrass, marjoram, mint, oregano, rosemary, thyme) have been thoroughly studied (Hedges and Lister, 2007), little research has been done to determine the effect of different storage temperature on VOCs.

The limited studies done in mango ginger have focussed only on physical attributes such as loss of moisture, shrivelling and loss of colour (Ravi and Aked, 1996). There is minimal literature available on the changes that happen to the VOC profile as a result of external factors which occur throughout the supply chain. Significant changes in the content and constitution of VOCs resulting from inadequacies in post-harvest processes (e.g., chilling injury during storage, mechanical injuries during handling) can detrimentally impact the final quality of the produce at the point of sale to the customer. This gap needs to be addressed by focused research in quantifying the losses that occur at different points in the supply chain cycle of mango ginger.

In addition to post-harvest practices, preharvest factors also greatly influence both the condition of the crop at time of harvest and the retention of morphological and organoleptic attributes during the crop's storage period. (El-Ramady et al., 2015) reports that many preharvest factors such as genotype, cultivar selection, climatic conditions such as temperature, humidity, light intensity, rainfall pattern, soil texture and fertility, nutrients, pest and disease management, and the maturity stage of the crops are known to affect storage quality of the produce. As such, the goal in managing preharvest factors should be to harvest the crop at the highest degree of quality and to sustain that quality throughout the storage period. The post-harvest quality of fresh horticultural commodities markedly depends upon the quality attained at the time of harvest.

The effect of different factors that influence the content and composition of essential oils are discussed next. Owing to the niche and non-commercial nature of mango ginger cultivation, there is a noticeable lack of literature focusing on the impact of biotic and abiotic factors on its aroma and sensory attributes, both in the preharvest and post-harvest stages. As such, literature has been obtained from similar work conducted in other aromatic plants to support the following discussion.

1.4.1 Preharvest factors

1.4.1.2 Biotic factors

Genetic and ecological factors and interactions between them have been found to affect the aroma profile of herbs in several studies (Pirbalouti et al., 2013a; Pirbalouti et al., 2013b; Ghasemi Pirbalouti et al., 2014; Pirbalouti et al., 2015, He et al., 2018, Turner et al., 2021). The strong relationship between chemical composition and genetic profile identified in *Ocimum basilicum* L. have led the way to a number of breeding programs and novel ways of cultivation (Liber et al., 2011). The chemical phenotype of aromatic plants (also referred to as the 'chemotype' (Sadeghi et al., 2015) is defined by the most abundant chemical produced by an individual and

can vary within the same species. Minor changes in the genetic make-up which have relatively small effects on the anatomy or morphology have been known to create significant changes in the chemotype. Explorations into the genetic make-up of thyme (*Thymus vulgaris*) have revealed that seven polymorphic chemotypes exist for this species which are largely indistinguishable in terms of appearance but have striking differences in the dominant compound (thymol, carvacrol, linalool, geraniol, sabinene hydrate (thuyanol), α-terpineol, or eucalyptol) of the essential oil (Keefover-Ring et al., 2009). Similarly, the ecological impact on chemotype has been studied in basil (*Ocimum basilicum*) to confirm the presence of different chemotypes depending on the location of cultivation (Patel et al., 2016). Several studies have shown that genotype and ecological factors and their interactions can affect some characteristics of herbs (Pirbalouti et al., 2013a; Pirbalouti et al., 2013b) such as wild and cultivated *Thymus daenensis Celak* and *Thymus vulgaris L* and mountain fennel (*Zaravschanica membranacea (Boiss.) M. Pimen*). Although limited studies have been conducted on the effect of genetic and ecological factors on mango ginger, the results from the above crops can be used as guidelines to understand the changes to its biochemical profile owing to the same VOCs being present in mango ginger.

1.4.1.3 Abiotic factors

In addition to chemotypes, aromatic plants are also classified into ecotypes due to variations in VOC composition arising from changes in environmental conditions (Nurzynska-Wierdak, 2012; Laribi et al., 2013). Differences in climatic conditions like day length, water status, temperature and light; cultivation conditions like sowing date, soil properties and soil type; agronomic practices like the dose of irrigation and fertilization; have all been linked to significant differences in VOC content (Dudai, 2005; Özgüven et al., 2008; Pirbalouti et al., 2013b; Rioba et al., 2015). The content of oxygenated monoterpenes and sesquiterpenes in basil were noted to be at their highest during winter (80.9 %) and lowest during summer (74.3 %) which were also reflected in the oil yield (0.8 % in winter and 0.5 % in summer) (Hussain et al., 2008). The place of origin, growing conditions, genotype, nutritional value of soil and chemo-types can also affect the essential oil yield as well as chemical composition of *Curcuma* sp. (Al-Qudah, 2017).

1.4.1.4 Phenological stages

Oil yield and the composition of VOCs are dependent on the stage of growth, as noted in studies conducted in *Tagetes minuta* (Moghaddam et al., 2007), *Cuminum cyminum* (Moghaddam et al., 2015a) and *Ocimum ciliatum* (Moghaddam et al., 2015b). In the case of *O. ciliatum*, essential oil yields (w/w %) were found to be 1.80, 1.30, 1.20, 1.13, 1.03 and 0.80 at budding, vegetative, initial flowering, full bloom, immature and ripen fruit, respectively. The oil content (w/w) of samples in *T. minuta* which were harvested at budding, full flowering and fruit set stages were also found to differ (1.55 %, 1.44 % and 1.0 % respectively). These changes have been attributed to modifications in the secondary metabolism and related enzyme activity along with the growth, development and harvesting maturity (Masango, 2005; Ghani et al., 2009; Casiglia et al., 2019) and underlines the importance of harvesting VOC storage organs like rhizomes at the right maturity.

1.4.1.5 Environmental stresses

The content of essential oils is often found to be enhanced as a defence response to environmental stresses, predominantly because of changes in the use of carbon for secondary metabolite production (Blanch et al., 2007). Severe environmental stresses like drought, extreme temperatures and pathogen infection can significantly reduce plant growth, and ultimately the rate of secondary metabolite production (Akula and Ravishankar, 2011; Copolovici et al., 2014; Timmusk et al., 2014). These metabolic reactions continue in storage organs like rhizomes even after harvest and can therefore affect the constitution of VOCs during post-harvest storage. For example, differences in drying methods have been found to significantly impact the quality of dried medicinal plants and both quality and quantity of essential oils (Buchaillot et al., 2009; Antal et al., 2011; Sellami et al., 2011; Huang et al., 2012). Studies conducted in lemon verbena found that the oil content decreased constantly when the duration of drying was increased and that most of the oil loss occurred at the beginning of the drying process, irrespective of the drying

temperature ranging from 30 °C to 60 °C (Shahhoseini et al., 2013; Argyropoulos and Müller, 2014).

As an exotic vegetable, mango ginger is imported into the UK, during which the rhizomes are exposed to different temperature conditions throughout its supply chain journey. An efficient supply chain that minimises the deterioration of post-harvest shelf-life quality is therefore an important consideration to ensure that the rhizomes maintain the concentration of phytochemicals and flavour components at the time of harvest, as well as throughout their storage period. Stability or unaltered biochemical activities of phytochemicals under prolonged storage conditions is a preferred quality for the production of pharmaceutical and nutraceutical products (Policegoudra et al., 2011). As mango ginger is rich in phytochemicals and high in demand in the food industry (Waman et al., 2021), there is an ongoing need to retain its nutritional attributes, flavour, and aroma aspects after harvest (Policegoudra and Aradhya, 2007). All key players in the supply chain cycle (e.g. farmers, wholesalers, retailers) must be familiar with the needs of a harvested product in order to maximise post-harvest shelf-life and the overall quality of the produce (Jobling, 2002).

1.4.2 Post-harvest factors

Post-harvest factors affecting plants vary greatly depending on the part of the plant that is used commercially. Even after harvest, agricultural produce remains alive and performs all the functions of a living tissue. There are a number of factors which affect these functions and ultimately the shelf-life of produce; the main ones being storage temperature and relative humidity. These factors are discussed below with the support of studies conducted on various aromatic plants with a view to explain their impact on VOCs to show that adherence to a systematic post-harvest regime suitable to the harvested produce is crucial in retaining the aroma, flavour, pungency, and colour of spices throughout their supply chain life cycle until they reach the end customer.

1.4.2.1 Storage temperature

Temperature management beginning from the point of harvesting to the point of consumption has been found to be an effective way to maintain the quality of harvested produce (Arah et al., 2015).Refrigerated storage has been known to slow down a number of factors that cause deterioration in harvested produce, such as ageing associated with ripening which commonly leads to tissue softening and changes to colour and texture, mainly because refrigeration is able to control the respiration rate of harvested produce. Oxidation of lipids, sugars and proteins in the cells occur as a result of respiration results in a loss of flavour and accelerates the deterioration of produce (Liu et al., 2019). There is a sufficient literature available which confirms that the respiration rate of agricultural produce is directly proportional to storage temperature and is also a key determinant of the transit and post-harvest shelf-life (Kader, 2003, Ansah et al., 2018; Duan et al., 2020; Salehi, 2020). Maintaining a low temperature regime is an essential and effective way of slowing bacterial growth, maintaining quality and minimising spoilage. Conversely, high temperatures can cause an increase in the rate of bacterial growth, enzyme activity and other chemical reactions. Low temperatures reduce respiration and ethylene production rates, water loss, pathogen growth, and decay incidence (Kader, 1992, Zainalabidin et al., 2019).

Temperature requirements vary among agricultural produce and even a short period of exposure to extreme hot or cold temperatures can cause a marked decrease in shelf-life and loss of quality. Hence standardised temperature management is essential to guarantee that the product arrives at its destination in the best possible condition. A temperature range between 0–5° C is recommended for fresh agricultural produce. However, a higher temperature of 12–13° C is required for some tropical crops as they are susceptible to chilling injury below 7 ° C which is accompanied by a loss of flavour and sprouting (Ravi and Aked, 1996).

1.4.2.2. Relative humidity

Water loss from stored produce is the most important attribute affected by a change in relative humidity, which ultimately has a severe detrimental effect on the quality of the produce. Water

loss also results in the loss of saleable weight and consequently the profit margin at the point of sale (Pinhero et al., 2009). The quality of fruits and vegetables is better retained at high relative humidity levels of 80 – 95 % as shown in studies conducted in litchi (Alférez et al., 2003), guava (Meng et al., 2016), brussels sprouts, zucchini, cabbage, cauliflower and celery (Van den Berg and Lentz, 1977; Zuo et al., 2021) owing to reduced trimming losses brought about by wilting and decay, as well as a reduced loss of juiciness and crispiness. Literature available on work done in tuber crops like potatoes also support the merits of maintaining high humidity levels during storage which resulted in significantly less weight loss, flattening and shrivelling (Sparks, 1973, Wang et al., 2016, Heltoft et al., 2016, Degebasa, 2020).

1.4.2.3. Mechanical damage (physical injury)

Careless handling of fresh produce is the main reason for mechanical damage and the resulting increase in the rate of physiological breakdown of stored produce. Moisture loss is the most visible after effect of mechanical damage. Studies conducted in potatoes identified that peeled potatoes lose three to four times as much weight when compared to unpeeled ones (Wilson et al., 1995). Mechanical damage can occur at any point during the supply chain cycle, i.e., during harvesting, transportation or produce display at retailers. This also becomes a serious food safety and economic problem as the produce will need to be consumed in a very short time period to prevent spoilage or infection of the wound by pathogens (Pinhero et al., 2009). The effect of post-harvest handling procedures such as peeling, cutting, slicing, shredding, trimming, coring, and the removal of protective epidermal cells has been studied in underground storage organs in different species, and confirmed that these cause severe physical stress causing internal discolouration, tissue breakdown followed by water loss, shrivelling and decaying (Brecht, 2002).

1.4.3. The effect of temperature on the post-harvest shelf-life of mango ginger

Although a cooler temperature regime during storage is preferred by many fruits and vegetables, the tropical origins of some crops like mango ginger make it susceptible to chilling injury at lower temperatures. One of the main consequences of chilling injury is the loss of VOCs, which

represents the most significant trait to determine the post-harvest quality of fresh herbs (Wang et al., 2015). This can limit the ability of post-harvest technologists to fully utilize temperature management to maintain the quality of the product (Brecht, 2003). Not many studies have been conducted on the effect of temperature on mango ginger, however there is abundant literature relating to other crops.

1.4.3.1 Fruits and Vegetables

A plethora of other studies support the theory that chilling injury caused by low temperatures induces a degradation of aroma and the underlying edible qualities of fruits and vegetables, mainly caused by a reduced enzymatic activity involved in volatile biosynthesis, consequently decreasing the abundance of almost all major volatile compounds(Zhang et al., 2011a; Aubert et al., 2014; Farneti et al., 2015). Cold storage conditions have been found to reduce aroma production in fruits such as peaches, kiwifruits, tomatoes, banana and mango (Raffo et al., 2008; Zhang, 2011; de Vasconcelos Facundo et al., 2012; Farneti et al., 2015; Günther et al., 2015; Purwanto et al., 2016) and the loss of aroma was found to be caused by disrupted cell membrane structure, membrane permeability and lipid degradation after cold storage. Loss of aroma esters and fatty acids was aggravated by the disruption of membrane lipid metabolism during shelf-life after long-term cold storage.

Studies conducted on coriander, cluster beans and beetroot have proven that the rate of respiration is directly proportional to storage temperature (Waghmare et al., 2013).These studies also showed that a three-fold increase in storage temperature (from 10° C to 30° C) lead to a five-fold increase in respiration rate, resulting in the production of enzymes reacting with the substrate to cause a reduction in the quality and shelf-life of the produce (Sandhya, 2010) .Other studies add support to the theory of a positive correlation between the rate of respiration and duration of shelf-life (Fonseca et al., 2002; Garcia and Barrett, 2002).

Different levels of chilling injury were observed to have different effects on VOCs in mango by (Nair et al., 2003) whereby a significant reduction in total aroma volatiles including

monoterpenes, sesquiterpenes, hydrocarbon, esters, and aldehyde production was observed in mango fruits stored at 0, 5, 10 or 15° C compared with fruit stored at 20° C. The study explains that chilling injury suppresses biosynthesis of fatty acids, which are the precursors of VOCs and reduce the activities of various enzymes involved in metabolic pathways for the conversion of acetyl CoA to volatile compounds. It also suggested that the reduction in aroma volatiles can be attributed to the suppression of ethylene biosynthesis. Chilling injury was also observed to delay the development of VOCs in apple and banana by impeding the breakdown of starch and acids (Yahia, 1994; Jiang et al., 2004).

Different storage temperatures have different effects on the physiology and biochemical changes resulting in changes of the volatile aroma profile and thereby reducing the shelf-life and sensory qualities of the harvested produce. Increasing the duration of low temperature storage (2–3 weeks at 1 - 4 ° C) in peach and nectarines significantly reduces the ability of the fruits to accumulate lactones, causing a reduction in VOC production (Sanchez et al., 2012). Fruits at 5 ° C were sensitive to chilling injury and had the lowest levels of volatile compounds, expressing low levels of compounds that contribute fruity notes such as esters and lactones. An increase in flesh tissue disruption was observed with a change in fatty acid profiles that resulted in altering the overall aroma profile (Zhang et al., 2011a). On the contrary, VOC production has also been observed to increase when the fruits are exposed to ripening (ambient) temperatures (Raffo et al., 2008; Sanchez et al., 2012; Aubert et al., 2014). Similarly, total volatiles in papaya was seen to reduce during cold storage (10° C) but restoring the fruit to 22° C results in a revival of the metabolic activities involved in respiration and ethylene production, pulp softening, and colour changes (Gomes et al., 2016).

Differences in storage temperature can result in membrane deterioration resulting in production of different volatiles (Deschene et al., 1991). In broccoli, temperature fluctuations during handling and distribution (4° C and 10° C) was seen to affect the aroma compounds in both raw and cooked produce, (Jacobsson et al., 2004). The study concluded that high temperatures resulted in high respiratory activity producing different volatiles, resulting in a larger number of total volatiles

being stored in broccoli florets. The associated development of aroma and off-flavours is enhanced by tissue disruption and cellular deterioration at higher temperatures (Chin and Lindsay, 1993; Tulio et al., 2002).

1.4.3.2 Spices and herbs

Lower temperature regimes have also been observed to significantly alter the aroma profile of herbs by changing the concentration of VOCs, mainly due to tissue degradation, related water loss, redox reactions and the triggering of the biochemical pathways like the mevalonic acid pathway. A study conducted by Cătunescu et al., (2016) using parsley (*Petroselinum crispum*), dill (*Anethum graveolens*) and lovage (*Levisticum officinale*) discovered cold storage at 4° C for 12 days resulted in an intensification of turpentine and mint notes in parsley, a decrease in intensity of musty notes in dill and an increase in spicy and balsamic aromas for lovage. The study observed both increases and decreases in VOCs as storage time increased; similar results were also reported in fresh cut pumelo wherein cyclopentyl-4-ethylbenzoate increased during storage, δ-cadinene and β-caryophyllene increased for three days and decreased at the end of storage (*Citrus maxima*) (Niponsak et al., 2011).

In cilantro (*Coriandrum sativum*), an initial increase of VOCs was detected for the first four days followed by a decrease when leaves were stored at 3° C (Loaiza and Cantwell, 1997; Fan et al., 2003) . This has been attributed to reduced rates of enzymatic biogenesis of aromatic compounds during the cold storage period. Similar studies using three different sweet basil cultivars revealed that storage temperature influenced the emission of volatiles, with much lower total volatile emission observed at 4 °C compared to 12 °C (Cozzolino et al., 2016). Cruz-Álvarez et al., (2013) identified that cooling or cooler temperatures (6 and 10 °C) enables maintenance of the respiration rate and ethylene production without causing significant changes in weight loss, total chlorophyll and external quality traits of peppermint, when compared to samples stored at room temperature.

In ginger, a temperature regime between -5° C and -20° C helps to preserve shelf-life and VOCs for up to 9 months when stored as whole rhizomes. Any decrease in temperature or increase in

duration results in a significant decrease in VOCs, free amino acids, unsaturated fatty acids and free sugars. Storage in the form of ground ginger is generally detrimental to overall shelf-life and customer acceptance due to a loss of flavour and aroma (Kim, 2003).

1.4.3.3 Mango ginger

Mango ginger being a tropical crop is susceptible to chilling injury caused by temperature abuse in the supply chain process, resulting in a loss of structural integrity and flavour compounds. Common symptoms of chilling injury are softening of tissues combined with water-soaked lesions browning, loss of the predominant mango flavour and rapid deterioration of physiological and antioxidant properties at 4 °C stored for three months (Policegoudra and Aradhya, 2007).

In the current study, chilling injury was induced in mango ginger by exposing the rhizomes to 2° C, over a period of ten days (Figure 3). Chilling injury symptoms were recorded during the first two weeks of storage. The intensity of the symptoms was recorded by visual observation. Symptoms of chilling injury were visible from the fourth day as slight browning around the cut edges of the rhizomes which covered 25 % of the area. Browning extended to 50 % of the area by the seventh day and 75 % over the next two days. The tissues also turned soft and dark brown by this point resembling water-soaked lesions. 100 % of the area was affected by the tenth day by which time the rhizomes when cut were soft, sticky and produced an off-aroma which aligned with the study conducted by Policegoudra and Aradhya (2007).

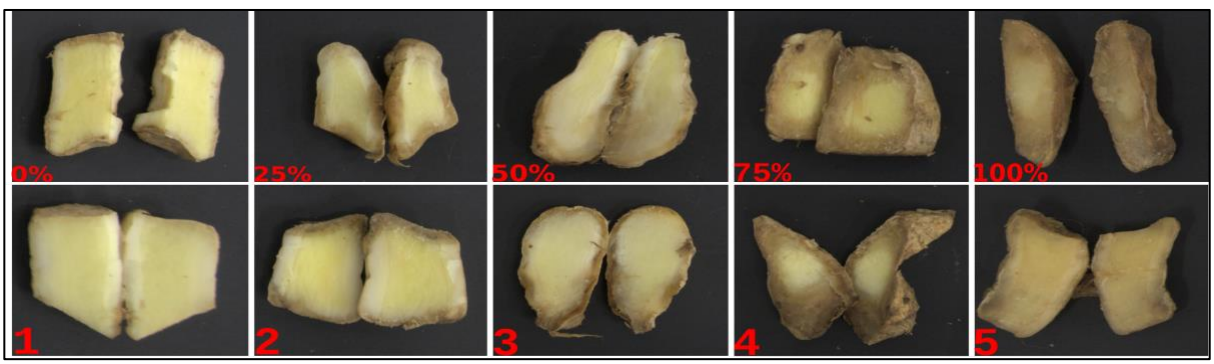


Figure 3 – Different stages of progressive chilling injury symptoms in mango ginger rhizomes stored in refrigerated conditions at 2° C. % values show area of rhizome affected by chilling injury; numbers denote stages of deterioration noted by visual observation.

1.5 The prevalence of drying as a post-harvest technique

Drying is one of the time-honoured techniques adopted for processing spices and other types of horticultural produce as part of post-harvest processing (Balasubramanian et al., 2016; Wang et al., 2013) providing longer shelf-life, product diversity and substantial volume reduction, which makes it popular in fruits, vegetables and spices alike. A general principle followed is to significantly reduce the initial moisture content of 55 – 85 % down to 8 – 12 % to prolong their shelf-life (Pruthi, 1992, Gunasekar et al., 2006, Balakrishnan et al., 2011). Drying also provides the added benefit of slowing down the growth of microbes and delaying physiological process that could alter the organoleptic properties of the harvested produce (Krokida and Marinos-Kouris, 2003).

Drying minimises the volume of the product (thereby reducing the amount of packaging material and subsequent transportation related waste) and makes it more suitable for further processing (Díaz-Maroto et al., 2003; Sagar and Kumar, 2010; Sellami et al., 2011). Over the years, there have been many advances in technology associated with commercial drying of food resulting in novel approaches to drying, such as microwave or ultrasound assisted drying, high electric field drying, heat pump drying and refractance window drying. All of these have improved the efficiency and efficacy of drying so as to reduce energy consumption whilst at the same time preserving the quality of the end product (Moses et al., 2014). Cost-effective and hygienic ways of preserving foods is of great importance given the prevailing insecurity in food supplies throughout the world. Novel technologies of food dehydration are a response to the latest consumer demands for dried products with superior quality while becoming more environmentally and economically sustainable. These improvements can increase the current degree of acceptance of dehydrated foods in the market (Maskan, 2001, Okos et al., 2018, Zhou and Wang, 2019, Wang et al., 2021). Conventional drying techniques adopted for spices and herbs range from solar drying (Özcan et al., 2005), hot air-drying (Demiray and Tulek, 2014), freeze-drying (Gümüşay et al., 2015) to microwave drying (Arslan et al., 2010). Different drying methods have different effects on the product used; the main causes of loss of volatile aromatic

compounds is the damage caused to the epidermis and cell wall structure (Lerdau et al., 1997; Lin et al., 1998; Yousif et al., 1999; Figiel et al., 2010, Szumny et al., 2010; Mirjalili et al., 2019).

As a majority of the aroma compounds in spices are volatile, it is important to select an appropriate drying regime that ensures a gentle removal of moisture with minimal impact on these compounds (Moses et al., 2014; Karam et al., 2016). However, enzymatic and non-enzymatic processes affected by these techniques have sometimes led to undesirable changes in the composition of phytochemicals, thereby affecting the aroma, flavour and texture of the harvested produce (Capecka et al., 2005). Currently, there is little evidence of scientific research conducted on mango ginger with respect to drying techniques used. However, several studies have taken place in other fresh herbs and spices which helps to compare the effectiveness of different drying techniques. These studies prove that the effectiveness of drying techniques is a function of three main factors, namely the type of horticultural produce, the drying method and the temperature regime followed during the processing. Microwave drying, oven drying and silica gel drying for ginger revealed that the major volatile compounds (zingiberene, β-phellanderene, β-sesquiphellandrene and geranial) retained in dried rhizomes were similar to those in fresh rhizomes, with marginally better retention of zingiberene using microwave drying (Huang et al., 2012).

Similar studies have confirmed that a high level of anti-inflammatory activity observed in fresh ginger due to the presence of non-volatile pungent components like gingerols was preserved in commercially processed dry ginger (Jolad et al., 2005). Bay leaf (*Laurus nobilis*) noted an increase in the eugenol content by 60 %; however oven drying at 45 °C produced better results in preserving the sensory characteristics when compared to freeze drying (Díaz-Maroto et al., 2002). On the contrary, both freeze drying and oven drying failed to capture over 80 % of the major aroma component (benzofuranoid) in dill (*Anethum graveolens*) and also created secondary aroma compounds (Huopalahti et al., 1985). Freeze-drying results in substantial losses of oxygenated terpenes and sesquiterpenes in spearmint (*Mentha spicata L.*) as it causes an expansion of the surface layer of cells (Díaz-Maroto et al., 2003).

In ginger, microwave drying at 60 °C was observed to be the optimum temperature to retain most of the aromatic qualities, when compared to 40 °C, where a longer duration of drying was required, and 70 °C where charring occurred (Hussain et al., 2009). Studies conducted on sage *(Salvia officinalis L.)* and thyme *(Thymus vulgaris L.)* found that both oven drying and freeze drying at 30 °C had minimal impact on volatile aromatic compounds; however, an increase in temperature to 60 °C caused a substantial loss of these compounds. The impact of changes to the aroma profile was more pronounced for thyme when compared to that of sage. The study concluded that loss of volatile constituents is dependent on both the drying conditions and the biological character of the harvested produce (Venskutonis, 1997, Hossain et al., 2010, Sellami et al., 2011).

Sub-optimal combinations of temperature and duration used in the drying process have been found to damage a number of bioactive compounds such as ascorbic acid, vitamin E, carotenoids, phenols and flavonoids along with side effects to physical attributes of the harvested produce such as broccoli and *Phyllanthus amarus* (Mrkìc et al., 2006; Nguyen et al., 2015). Changes in the quantity of certain volatile compounds present in basil and ginger (Baritaux et al., 1992; Bartley and Jacobs, 2000) or the formation of new compounds in spices have in some cases been observed after drying, probably as a consequence of oxidation reactions, hydrolysis of glycosylated forms or the release of substances following the rupture of cell walls (Huopalahti et al., 1985, Sellami et al., 2012 ,Kripanand and Guruguntla, 2015). As a result, drying has been found to bring about a reduction in the overall aroma of the spice and in some cases give rise to a secondary aroma (Díaz-M*aroto* et al., 2002).

1.6 Production of Volatile Organic Compounds by plants

Plants produce a diverse spectrum of essential oils known as VOCs or ethereal oils, extracted from various aromatic plants as well as from different parts of the plants such as flowers, roots, fruits, leaves and seeds (Guenther and Althausen, 1948). Studies on plant volatiles such as isoprenoids reveal that they are produced as plant protective and signalling molecules, usually induced due to stress factors such as oxidation (which causes lipid breakdown), extreme high temperatures and pathogen attack (Leitner et al., 2008; Vickers et al., 2009). Lipid derived volatiles such as (Z)-3-hexanol, (E)-2-hexanal and (Z)-3-hexenlyl acetate and linalool are credited with the inhibition of pathogen growth (Kishimoto et al, 2006).

Plant volatiles are low-molecular-weight compounds (below 300 Da) and can typically be divided into three major compound groups: terpenoids/isoprenoids, phenylpropanoids/benzenoids and fatty acid derivatives. The largest class of plant volatile compounds are mainly represented by isoprene (C5), monoterpene (C10) and sesquiterpenes (C15) compounds. The synthesis of VOCs takes place through a number of major biochemical pathways but terpenoids, the most common compound group observed in mango ginger are synthesised from the universal five carbon precursors including isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in the mevalonate or non-mevalonate pathway. During their synthesis, various forms of enzymatic modifications such as hydroxylation, acetylation and methylation take place. As the proportion of individual compounds in the oil composition varies considerably, the characteristic aroma is the result of a combination of all the released volatile components (Nagegowda, 2010).

Although less abundant, trace components like α-thujene, eucalyptol, menthol and α-humulene are just as important, as they also contribute to the character and natural aroma of the oil, as noted in studies in coriander (Pourmortazavi and Hajimirsadeghi, 2007). Both hydrocarbons and oxygenated compounds in oils are responsible for the characteristic aroma and flavours of spices and all together, this adds to the diversity of the volatile composition (Dudareva et al., 2006).

1.6.1 Major aroma components identified in mango ginger

Experiments studying the aroma composition in mango ginger date back to the 1940s, where (Dutt and Tayal, 1941) identified ocimene as a major component within the aroma profile of essential oil of mango ginger rhizomes, in addition to linalool, linalyl acetate and safrole. These compounds have been identified to possess floral and woody odour descriptors. A number of studies have determined a mixture of volatile and non-volatile components to contribute to the characteristic aroma and flavour in mango ginger. Jain (1964), Ahuja and Nigam, (1971) reported that the major aroma compounds in mango ginger were curcumene, pinene, camphor and α-turmerone. Using the essential oil of mango ginger and gas liquid chromatography (GLC) analysis, Gholap and Bandyopadhyay (1984) reported that 3-carene and cis-ocimene were responsible for the raw mango flavour along with α-pinene. Similar results were obtained from GLC studies on latex extracted from green mangoes, explaining the similarities in aroma observed between mango ginger and raw mangoes. Terpene compounds have been identified as one of the most essential compounds of the steam volatile oils of mango ginger (Govindarajan and Stahl, 1980).

In total, around 130 chemical constituents have been reported in fresh mango ginger rhizome and out of these, 121 have been identified (Jatoi et al., 2007). Jatoi et al., (2007) identified the composition of fresh and dried mango ginger to comprise: myrcene (88.6 %), ocimene (47.2 %), α -turmerone (29.12 %), Z-β-farnesene (21.9 %), guaia-6-9-diene (19.8 %), *cis*-β-Ocimene (18.8 %), *cis*-hydro-ocimene (18.79 %), *trans*-hydro Ocimene (15.94 %), α-longipinene (14.8 %), α-guaiene (14.5 %), linalool (13.37 %), β- curcumene (11.2 %) and turmerone (10.8 %).

Mustafa et al., (2005) extracted the essential oil of mango ginger using steam distillation, confirming the presence of twenty-six compounds which comprised the total volatile content, of which twenty-four components comprised 96.1 % of the oil. The oil was found to be a mixture of monoterpenes, sesquiterpenes, alcohols, ketone and esters. The sesquiterpene fraction consisted of hydrocarbons Z-β-farnesene (21.9 %), α-longipinene (14.8 %), α-guaiene (14.5 %)

and guaia-6-9-diene (19.8 %). The predominant monoterpenes identified were camphor (5.5 %) and thymol (4.9 %), the latter was observed to be a characteristic compound of the mango ginger extract.

The raw mango flavour and the compounds that contribute towards it have been observed by MacLeod (1982) in Venezuelan mango fruits. Using gas chromatography mass spectrometry (GCMS) combined with both electron and chemical ionization, reported that the important constituents included α-pinene, 3-carene, limonene, γ-terpinene, α-humulene, β-selinene, acetophenone, benzaldehyde and dimethyl styrene. Here, 3-carene was identified as contributing the most to the raw mango flavour, comprising 26 % of the aroma composition respectively.

Rao et al. (1989) similarly identified the presence of 3-carene in mango ginger, reporting that it imparted a floral and leafy mango aroma along with *cis*-ocimene and α-pinene. Within this study, Rao et al. (1989) used GCMS to identify sixty-eight compounds in mango ginger which comprised a mixture of hydrocarbons, alcohols, aldehydes, ketones and esters. Their investigation also considered *cis*- and *trans*- hydro-ocimene, ocimene and myrcene to be the major aroma character impacting compounds of mango ginger. Successful isolation of curzerenone and epicurzerenone was also completed, both of which were also reported to be present in *Curcuma zedoaria* (Hikino et al., 1975). A similar mixture of compounds has been observed in turmeric and mango by Govindarajan and Stahl (1980), where *cis*-ocimene imparted the flavour of raw green mango, while α-pinene and 3-carene were reported to impart floral and leafy mango aromas. This was supported further to confirm the sesquiterpene known as curcumene present in turmeric is also observed in mango ginger (Jain, 1964; Ahuja and Nigam, 1971) .

Analysis of essential oils from dried rhizomes by GC and GC-MS analysis showed the presence of twenty two compounds accounting for 91.47 % of the rhizome oil (Singh et al., 2003; Singh et al., 2002). Among them the major constituents found was myrcene (80.54 %), followed by β-pinene, β-(E)-ocimene and perillene.

As discussed earlier, chemical variation has been observed when different parts of the same plant are investigated and this was observed by Padalia *et al*., (2013) when the essential oil of the leaves and rhizomes of mango ginger were analysed by gas chromatography flame ionization detector (GCFID) and GCMS, revealing a sharp qualitative and quantitative variation in their essential oil components. Here, fifty-seven compounds from both leaves and rhizomes were identified including furanosesqueterpenoids such as epi-curzerenone (10.76 %), curzerenone (9.53 %), curzerene (3.95 %) and furanogermenone (1.77 %)) and monoterpenoids including camphor (17.9 %), isoborneol (7.3 %), camphene (3.57 %), borneol (1.87 %) and camphene hydrate (1.25 %). These compounds were identified as the major fractions of the leaf essential oil composition. The rhizome oil components were dominated by monoterpenoids (97.72 %) namely myrcene (88.84 %), β-pinene (3.74 %) and β-(E)-ocimene (2.61 %), these compounds were reported to be responsible for the characteristic raw mango aroma of *Curcuma amada*. Compounds such as furanosesqueterpenoids, isoborneol and borneol were found to be the exclusive constituents in the leaf essential oil. This is the only reported study so far on the essential oil from leaves of *Curcuma amada*.

Quantitative analysis of the chemical composition of mango ginger essential oil using GCMS analysis conducted by George et al (2015), identified a profile of 17 phytochemical compounds were determined to constitute the aroma composition including caryophyllene, allo-aromadendrene, α-pinene and farnesene. Of these seventeen compounds, β-myrcene and β-pinene were two major components and have been shown to have antimicrobial activities.

The rhizomes of Malaysian *Curcuma mangga* Val. & van Zijp were discovered to have similar essential oil components to those identified in *Curcuma amada Roxb*. and these may be responsible for conferring a mango-like aroma. The essential oil obtained by hydro distillation of the rhizomes of *Curcuma mangga* Val. & van Zijp was analysed by capillary GC and GCMS. Forty-four components were identified, and monoterpene hydrocarbons were observed to be most dominant, accounting for 91.2 % of the oil. The oil was identified to comprise 12 monoterpene hydrocarbons (91.7 %), 22 oxygen-containing monoterpenes (3.4 %), seven sesquiterpenoids

(1.1 %) and three miscellaneous compounds (0.2 %). Myrcene was clearly dominant, representing 78.6 % of the oil whereas other quantitatively significant constituents included α -pinene (2.9 %), β -pinene (3.7 %) and β-(E)-ocimene (5.1 %). Interestingly, it was found that these monoterpene hydrocarbons like myrcene and (Z)-ocimene were also found among the volatiles of mango, in two cultivars, Alphonso and Baladi, from Egypt (Engel and Tressl, 1983). This study showed that the composition of *C. mangga* oil shares a number of volatile terpenes with *C. amada* reported by (Choudhury et al., 1996b).

Kamazeri (2012) analysed the essential oil obtained from Malaysian *Curcuma mangga* Val. & Zijp (*Zingiberaceae*) rhizomes by GC and GCMS, identifying 97 constituents, comprising 89.5 % of the total oil composition. Here, the major compounds were identified as myrcene (46.5 %) and β-pinene (14.6 %). Collating all the data that has been generated so far, it is clear that myrcene as a major component in the essential oils, as reported by (Choudhury et al., 1996a). The study was conducted using the essential oil, which was obtained by hydro distillation of the fresh rhizomes of *Curcuma amada Roxb*. (Zingiberaceae) growing in the plain districts of North-eastern India, and later identified using GCMS. Of the identified and isolated nine compounds, myrcene was found as the major (88.6 %) component in the essential oil.

A recent study on the chemical composition and antioxidant potential of essential oil from the rhizome of mango ginger by Tamta et al. (2016) found that the freshy crushed rhizome when extracted with several solvents and subsequent GC-MS analysis yielded several compounds of which, β-myrcene was found to be the highest constituting (40.2 %) followed by 10 % curcumene. This study also agreed with that of Rao et al. (1989) that the cis-and trans hydro-ocimene, ocimene and myrcene were found to be the major compounds of the volatile oils which indicate that a mixture of components present in raw mango and turmeric contribute to the characteristic aroma in Mango ginger.

1.7 Conclusion

Mango ginger is exported as an exotic vegetable and is exposed to different temperature and moisture conditions during its supply chain. Whether freshly harvested rhizomes kept in these conditions can still retain the correct balance of VOCs, which imparts its characteristic flavour and aroma until they reach the end customer, remains understudied and not understood. Although extensive work on post-harvest processing has been done in turmeric and ginger, mango ginger seems to be an untapped and underutilized spice in the Zingiberaceae family (Policegoudra et al., 2011; Saipriya et al., 2017; Ravindran et al., 2012). Most of the information relating to the traditional uses of mango ginger is scattered with some of this only being available in local languages which can be difficult to access (Jatoi et al., 2007). Although many research findings have confirmed that mango ginger has numerous valuable properties including antioxidant, antibacterial, antifungal and anti-cancerous, little attention has been given to the changes in the phytochemicals, especially volatile compounds in the supply chain process (Shahrajabian et al., 2019).

There is also a lack of sufficient literature around post-harvest processing of mango ginger rhizomes and the further utilization of the VOCs in the food and pharmaceutical industries. Optimisation of a suitable drying technique, the effect of different drying regimes on volatile aromatic compounds and organoleptic properties for mango ginger have not been researched upon, owing to the niche nature of this exotic spice. If an optimum temperature range and drying technique can be identified which helps to retain the flavour and aroma of mango ginger, this study is expected to address the knowledge gap surrounding the feasibility of commercially extracting and using these phytochemicals. This will act as a steppingstone for further studies which can focus on utilizing them as natural flavourings spice in food, drinks, confectioneries and in the pharmaceutical industry.

This study therefore aims to evaluate the effect of different storage temperatures and two drying techniques – hot-air drying and freeze drying, on VOCs of mango ginger.

1.8 Aims of the study

A. To evaluate the effect of different storage temperatures on the VOCs of mango ginger at critical time points across its shelf-life period.

and

B. To evaluate the effect of different processing techniques on the VOCs of mango ginger at critical time points across its shelf-life period.

1.9 Objectives of the study

A1. To determine the effects of varying storage temperatures on the volatile aroma profile of mango ginger at critical time points across a one-month shelf-life period (Chapter 2);

A2. To determine the effects of different storage temperatures during storage conditions and their impact on the organoleptic properties of mango ginger (Chapter 2);

B1. To determine the effects of different processing techniques on the volatile aroma profile of mango ginger during a three-month shelf-life period (Chapter 3);

B2. To determine the effects of different processing techniques on the volatile aroma profile and their impact on the organoleptic properties of mango ginger (Chapter 3);

CHAPTER 2

2. Changes in VOC and sensory properties of fresh mango ginger rhizomes stored at different temperatures and durations

2.1 Introduction

Policegoudra and Aradhya (2007) explored the effect of different storage temperatures on the physiological changes and antioxidant activities in mango ginger to determine whether maintaining an optimum balance between storage temperature and duration has a beneficial impact on physiological and biochemical changes taking place within plant cells, thereby ensuring a better quality produce with a longer shelf-life , while retaining most of its inherent nutritional and organoleptic properties. Rhizomes were stored for 120 days under three storage temperatures (25 °C, 14 °C or 4 °C). Changes in total phenolics, antioxidant activity, pH, titratable acidity, total soluble solids, total sugars, reducing sugars and protein content were recorded throughout the shelf-life period. The effect on the shelf-life of the rhizome, moisture content, sprouting and other post-harvest losses such as chilling injury were also examined. The study found that storage at ambient temperature (25 °C) stimulated moisture loss, increased the total soluble sugars, phenolics and antioxidant activity of the rhizome, consequently reducing its shelf-life to three months, when compared to samples stored at 14 °C. Similarly, chilling temperature (4 °C) was also seen to negatively affect the shelf-life of the rhizome and was characterised by an initial accumulation of total soluble sugars, total sugars and phenolics which later decreased rapidly, consequently limiting the shelf-life period to 30 days. On the other hand, storage at 14 °C was found to be optimal in maintaining the biochemical, antioxidant and the mango flavour retention properties. This alleviated excess moisture loss, chilling injury, sprouting and extended the shelf-life up to 4 to 5 months.

The results of the above study point to the fact that the post-harvest quality of mango ginger rhizomes is a function of storage temperature and time, by having a direct impact on the physiological activities during the post-harvest period. Changes in these activities impact

phenolic concentrations, sugar and starch content and have a direct impact on secondary metabolites produced within the rhizome. These ultimately result in the alterations of the aroma and flavour compounds present in them. A similar behaviour can been seen in ginger (Variyar et al., 2007), where rhizomes exposed to controlled Gamma radiation prevented sprouting and extended the shelf-life of ginger when stored at ambient temperature.

Although a number of studies have been conducted to identify the major chemical constituents of the essential oils in mango ginger, the effect of storage time and temperature on the essential oil components and its related changes in sensory perception and organoleptic properties during the shelf-life period have not been widely studied. The first phase of this study therefore seeks to identify changes in the sensory perception of aroma and flavour compounds in mango ginger, when stored at three different temperatures for a duration of one month.

2.2 Materials and Methods

2.2.1 Preparation of samples

Experiments were carried out to determine the impact of different storage temperature conditions on fresh mango ginger rhizomes: the first one relying on instrumental analysis and the second one on sensory analysis. Bagged mango ginger rhizomes were purchased from the Western International wholesale market in Hayes, Middlesex UK. Each microperforated plastic bag contained 5 kg of fresh mango ginger and as per the labels on the bags, the plants were grown in Mumbai, India. Twelve bags were purchased on the day they arrived in the wholesale market and each bag was used as a replicate. They were then transported under ambient conditions and stored in the pilot plant at the University of Reading (under ambient conditions) until further sorting for differential temperature treatments.

An intake analysis was then performed to remove rhizomes with undesirable characteristics (e.g., those that were undersized, those with physical damage, signs of pest and disease attack and brown spots as a result of chilling injury). The remaining samples were grouped into 2 kg lots (each lot was treated as a replicate) and stored in separate plastic crates under three different storage temperatures as shown below:

- 8 °C (T1) in a temperature-controlled storage cabinet
- 15 °C (T2) in a temperature-controlled storage cabinet
- 22 °C (T3) in a temperature-controlled storage cabinet under ambient conditions (Control)

Each of the storage cabinets had minor differences in temperature (+ or – 1 °C), which made the cabinets colder towards the rear, gradually becoming warmer towards the front. In order to ensure uniform exposure to temperature, the crates were spread across the front, middle and rear sections of each storage cabinet. Light intensity was controlled by covering the crates with paper sheets.

Samples were arranged in front, middle and rear end for a period of one month in a Randomised Block design. The experiment was designed such that sensory evaluation and instrumental analysis could be done on the same day at specific intervals during the one-month storage period. These specific points in time are shown below.

- Day 0 – the date on which the samples arrived at the University of Reading
- Day 1 (D1) – the day following day 0
- Day 15 (D15) – the day falling two weeks after day 0
- Day 29 (D29) – the day falling four weeks after day 0

Sensory and instrumental analysis of VOCs were conducted on day 1, 15 and 29. Four rhizomes were collected from the front, middle and rear end of the crates. Selected rhizomes were then mixed up and washed thoroughly in tap water. A smooth bristled brush was used to clean off the dirt, dry scales and soil particles. Cleaned and edible rhizomes were used for sensory evaluation. Two replications per sample were also collected from each randomised block for instrumental analysis.

2.2.2 Evaluation of changes in the volatile aroma profile of fresh mango ginger stored at different temperatures by Instrumental Analysis

The samples stored under different temperatures were analysed for the changes in their volatile profile across different time points. Automated Headspace Solid Phase Microextraction method (HS-SPME) was used as described by Morales-Soto et al. (2015) for the identification of the volatile aroma compounds. The method was modified by several initial trials (by exposing the samples to different temperature treatments such as 22 °C, 30 °C, 35 °C and 37 °C) so as to optimise extraction of volatiles from mango ginger. For GCMS analysis, calcium chloride and the alkane standard C6–C25 (100 μg/mL) in diethyl ether were obtained from Merck (Poole, UK).

2.2.2.1 Solid Phase Microextraction (SPME) followed by GCMS

Two rhizomes from the randomised blocks were selected for the analytical study. 100 milligrams of freshly grated samples were placed in a 15mL SPME vial with a fitted screw cap. A saturated solution of calcium chloride (1 mL) was added to the samples to prevent unintended release of volatile compounds due to enzymatic reactions. Analysis was carried out by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA, USA). The SPME fibre stationary phase was composed of 75 µm divinylbenzene/Carboxen™ on polydimethylsiloxane, Supelco (Bellefonte, PA, USA). Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C.

After extraction, the SPME device was inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column HP-5MS (30 m × 250 µm × 0.25 µm thickness) (Agilent, Santa Clara, CA, USA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C and 6 min at 250 °C isothermal. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature of the injector, interface and detector was 250 °C and the sample injection mode was split less. Mass spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 m/z and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system. Two replicates were prepared for each sample totalling six replicates for each storage temperature treatments. An internal standard of 10 µL (100 ppm) Propyl propionate was added into each sample.

For data processing, the spectrum from data was controlled and stored by the HP G1034C Chemstation system. VOCs were identified by comparison of each mass authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading) with The National Institute of Standards and Technology (NIST) databases, (NIST/EPA/NIH Mass Spectral

database, 2011), spectra published in other literature and confirmed in many cases by comparison of their retention indices as shown in ADAMS library. To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound, using the retention times of a homologous series of C6–C25 n-alkanes and by comparing the LRI with those of authentic compounds.

2.2.3 Evaluation of changes in the volatile aroma profile of fresh mango ginger stored at different temperatures by Sensory Analysis

Specific sets of sensory descriptors for mango ginger were established using an expert panel of eleven sensory assessors, who had been trained in accordance with ISO 8586:2012 (the ISO standard for sensory analysis) and ISO 11132:2012 (the ISO standard for performance monitoring). The trained sensory panel at the Sensory Science Centre (University of Reading, n = 11; 10 female and 1 male) had a minimum of 6 months' worth of experience in sensory evaluation, with some of them having up to 8 years of experience.

Samples were presented in a random, coded fashion over the course of half-hour sessions on two consecutive days. Panellists were asked to grate the samples to release the aroma compounds and taste them to produce as many descriptive terms as seemed appropriate. Assessors discussed, with the aid of a facilitator, the various sensory attributes associated with the appearance, odour, mouthfeel, taste, flavour and after-effects of fresh rhizome samples. Reference standards were used where appropriate to ensure agreement of the descriptive terms chosen. For example, as raw mango ginger flavour has an exotic aroma resembling that of mangoes and turmeric, raw mangoes and turmeric were purchased from the local market and presented to the sensory panel as a reference material for sniffing. Once a consensus set of descriptors for various attributes was established, a formal sensory assessment was conducted. Two replicates from each sample set kept at three different temperatures were presented to the panellists.

Quantitative sensory measurement took place in separate sensory booths, each fitted with a computer screen and mouse. The software used for sensory data acquisition was Compusense

(Compusense Inc., Guelph, Ontario, Canada). The samples presented to the panellist included a mixture of rhizomes which were randomly selected from three different positions of the temperature-controlled storage unit (front, middle and rear positions). The selected samples were washed, cleaned and placed in labelled saucers. The samples were presented to the assessors in randomised order. So as to avoid lingering tastes interfering with successive scoring of samples, a two-minute break was provided between scoring each sample. Warm water and wipes were provided for cleaning the assessor's fingers and the graters used after each scoring and assessment, so as to avoid aroma being carried over to subsequent samples. Full fat yoghurt, crackers and cold water was also provided for palate cleansing between each sample. Owing to the strong aftertaste of the rhizomes, it was decided to conduct only one replication of each of the three temperature treated samples per day i.e., one set of samples were scored each day (day 1 and day 2, day 15 and day 16, day 29 and day 30). Each sample was presented and assessed twice by each of the 11 panellists, and averaged for sensory analysis.

The strength of each attribute for each sample was recorded by the assessors on an unstructured line scale (10 cm, scaled 0-100). This led to an agreed profile comprising of six appearance terms, six aroma terms, seven flavour / taste terms, four mouthfeel terms and five aftertaste terms (Table 1). Each panellist was provided with an isolated sensory booth within the Sensory Science Centre at the Department of Food & Nutritional Sciences, University of Reading. All evaluation sessions were carried out under artificial daylight conditions in an air-conditioned room (~22 °C).

Table 1 – Lexicon developed by sensory panellists for fresh mango ginger samples (n=11).

Category	Attribute	Anchor point	Agreed definitions
Appearance	Shiny	Low to High	Smooth outer skin with a fresh appearance
	Scaly		Presence of thin dried paper like flakes on the skin
	Presence of sprouts		Presence of germinating buds (similar to 'eyes' in potatoes)
	Shrivelled		Dried and shrivelled outer skin
	Distinct core	Light to Dark	Appearance of two distinct coloured areas within the rhizome
	Core colour	White to Yellow	Light yellow to greenish core, resembling that of raw mangoes
Aroma	Green fresh	Mild to Extreme	Emitting the smell of fresh cut grass
	Floral		Emitting the smell of flowers
	Green mango		Emitting the smell of freshly cut raw mango
	Soapy		Emitting the smell of a bar of soap
	Piney		Emitting the smell of crushed pine leaves
	Mild turmeric		Emitting the smell of earthy fresh turmeric
Flavour/taste	Bitter	Mild to Extreme	Unpleasant, sharp or pungent taste on the tongue
	Raw mango		Resembling the flavour of a freshly cut raw mango
	Mild turmeric		Resembling the flavour of a freshly cut raw turmeric
	Green fresh		Emitting the smell of fresh cut grass
	Soapy		Soap like and bitter sensation on the tongue
	Sweet		Pleasant taste associated with sugary food
	Piney		Resembling the flavour of a freshly crushed pine leaf
Mouthfeel	Crunchy	Less to High	Brittle, fresh sensation on the tongue and teeth similar to when eating fresh fruits and vegetables
	Fibrous		The feel of strands on the tongue and teeth when samples are chewed
	Moist		The feel of juiciness when samples are chewed
	Warming		The sensation of increased temperature within the mouth while chewing for a slightly prolonged time interval
Aftertaste	Bitter	Mild to Extreme	An unpleasant, sharp or pungent taste on the tongue
	Mouth drying		The feeling of a dry mouth
	Salivating		Excessive saliva production in the mouth as a result of bitterness; also associated with sharp 'piney'ness
	Soapy		Soap like and bitter sensation on the tongue
	Throat burn		Sensation of throat 'catching at its back' due to heat sensation

2.2.3.1 Statistical Analysis

The relative abundance of VOCs was calculated from the data collected by SPME GCMS analysis. Quantitative data for each compound identified in the SPME GCMS analysis were analysed by both one-way and two-way analysis of variance (ANOVA) and principal component analysis (PCA) using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine the sample means that differed significantly ($p < 0.05$) between storage temperature and storage duration of the fresh rhizomes.

Each sample was presented and assessed twice by each of the 11 panellists, and averaged for sensory analysis. SENPAQ version 3.2 (Qi Statistics, Reading, UK) was used to carry out ANOVA and PCA of sensory panel data. The means for the sensory data were taken over the assessors and correlated with the means from instrumental data via principal component analysis (PCA) using XLSTAT.

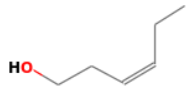
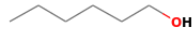
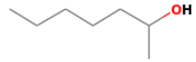
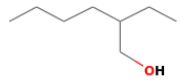
2.3 Results and Discussion

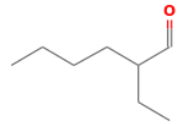
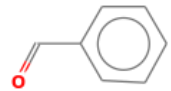
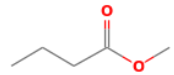
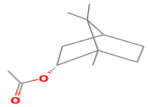
2.3.1 Volatile Composition

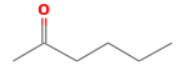
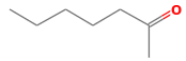
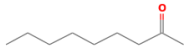
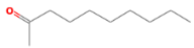
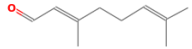
Forty-four compounds in total were identified in the headspace (Table 2). These consisted of 22 monoterpenes, 5 sesquiterpenes 6 alcohols, 5 ketones, 3 aldehydes and 3 esters. In terms of relative abundance, the major volatile groups in mango ginger were identified as terpenes (monoterpenes and sesquiterpenes), ketones and alcohols, when compared to that of aldehydes and esters (Table 3). Twenty-two monoterpenes were identified during this study, of which the mean relative abundance values of the monoterpenes myrcene (995.4), β-(E)-ocimene (200.9), β-pinene (160.8), eucalyptol (45.5), epoxy myrcene (27.4) and α-pinene (20.9) made them stand out as the major compounds (having a relative abundance value of more than 20) contributing to the volatile aroma profile.

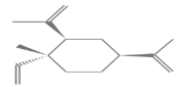
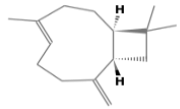
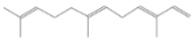
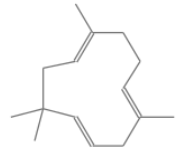
These results were found to align with a number of reported studies (Rao *e*t al., 1989; Jatoi et al., 2007; Kamazeri, 2012; Tamta et al., 2016) which show that monoterpenes dominated the total composition of terpenes in mango ginger. *Cis*-ocimene has been previously reported as being responsible for the green mango aroma, and α-pinene and car-3-ene responsible for floral and leafy mango aromas (Rao et al., 1989). Ocimene, *cis*- and *trans*- dihydro ocimene and myrcene were also reported to impart an overall raw mango and turmeric flavour (Govindarajan and Stahl, 1980; Ramteke et al., 1981). Despite the relatively lower concentrations of aldehydes and esters, they are considered to be associated with green, floral, citrus and fresh odours (Turner et al., 2021) and therefore important components of the overall profile.

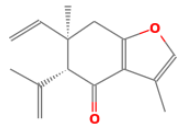
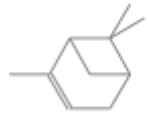
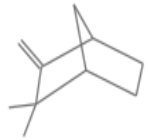
Table 2– Volatile compounds identified from fresh mango ginger rhizomes.

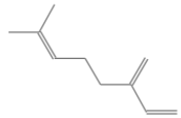
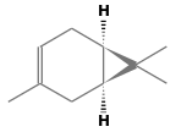
Compounds	Material / Source	Active ions	LRI expt (a)	ID (b)	Molecular Weight (g/mol)	Organoleptic properties	Molecular structure	Other reported references in mango ginger (c)
1-pentanol	Fresh rhizome	42, 55, 70	762	(A)	88.15	fruity aroma		
(Z)-3-hexen-1-ol	Fresh rhizome	41, 55, 67, 82	850	(A)	100.16	fresh cut green grass		
hexanol	Fresh rhizome	43, 56, 69, 84	863	(B2)	102.17	fatty, fruity, woody		
2-heptanol	Fresh rhizome	45, 55, 83	894	(A)	114.19	fresh, green, citrus		
2-ethyl hexanol	Oil from fresh rhizome	29, 41, 57, 70, 83, 98	1029	(B4)	130.2	citrusy, fatty		7,8

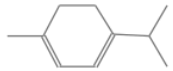
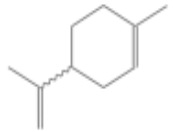
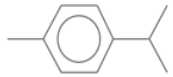
2-nonanol	Oils from fresh and dried rhizomes	45, 55, 69	1099	(A)	144	waxy, soapy musty with green fruity and dairy nuances		9
hexanal	Fresh rhizome	44, 56, 74, 82	801	(A)	100.16	fruity, grassy		
2-ethyl- 2-hexenal	Fresh rhizome	41, 55, 69, 83, 98	846	(A)	128.21	Strong odour		
benzaldehyde	Fresh rhizome	51, 77, 106	952	(A)	106.12	cool, woody, minty, green piney		
methyl butanoate	Fresh rhizome	42, 59, 74, 87	721	(A)	102.13	fruity apple like odour		
bornyl acetate	Fresh rhizome	43, 95, 121, 136, 154	1296	(A)	196	green, woody		11

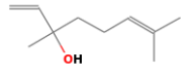
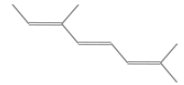
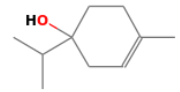
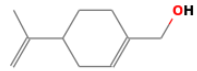
isopropyl tetradecanoate	Fresh rhizome	43, 60, 73, 102, 129, 228	1825	(A)	270.5	odourless		
2-hexanone	Fresh rhizome	28, 43, 71, 85, 100	791	(B1)	100.16	fruity, buttery		
2-heptanone	Fresh rhizome	45, 55, 83	889	(A)	114.19	cheesy, fruity		
2-nonanone	Fresh rhizomes	43, 58, 71	1091	(A)	142.2	fruity, cheesy		9,11
2-decanone	Fresh rhizome	43, 58, 71, 156	1192	(A)	156	floral, fermented		
2-undecanone	Oils from leaves, fresh and dried rhizomes	43, 58, 71	1293	(A)	170	balsamic, woody, fresh pine		11

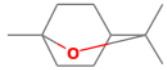
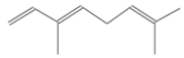
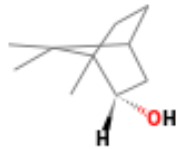
β-elemene	Fresh rhizome	41, 53, 68, 81, 93, 107, 121, 147, 189	1407	(B7)	204	herbal, waxy		9,11
E- caryophyllene	Oils from leaves, fresh and dried rhizomes	41, 55, 69, 93, 133, 147, 161, 189, 204	1445	(A)	204	floral		1,2,3,4,7
β -farnesene	Oils from leaves and fresh rhizomes	41, 69, 93, 120, 133, 160, 204	1452	(A)	204	woody, clove		1
α-humulene	Oils from fresh rhizomes	41, 80, 93, 107, 121, 147, 204	1460	(A)	204	woody, bitter, spicey		3,7

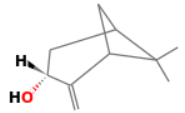
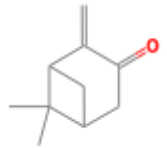
curzerenone	Fresh rhizome	94, 122, 230	1627	(A)	230	earthy, turmeric -like		9,11
α-thujene	Fresh rhizome	41, 77, 93, 136	924	(A)	136.23	sweet, woody, piney, earthy		
α-pinene	Oil from fresh leaves and rhizomes	77, 93, 105, 121, 136	932	(A)	136	woody, herbal camphor, cooling, minty		2,3,4,6,7,8
camphene	Oil from fresh leaves and rhizomes, dried rhizomes	53, 67, 79, 93, 107, 121, 136	946	(A)	136	almond like		1,3,4,5,7,8,9,10

sabinene	Fresh rhizome	41, 77, 93, 136	969	(B3)	136	dry, woody, piney, resinous		3
β-pinene	Oil from fresh leaves and rhizomes	41, 69, 79, 93, 121, 136	985	(A)	136	mango, spicy woody, balsamic fresh, sweet, citrusy		1,2,3,4,5,8
myrcene	Oil from fresh leaves and rhizomes	41, 69, 93, 121, 136	988	(A)	136	turpentine like		1,2,4,5,7,10
δ-3-carene	Oil from fresh rhizomes	65, 77, 93, 105, 121, 136	1008	(A)	136.23	citrusy, fatty		6,9,10

α-terpinene	Oil from fresh leaves and rhizomes	29, 43, 57, 70, 83	1024	(A)	130.23	terpenic, woody odour		1,2,3,4,5,6,7,8
limonene	Oil from fresh leaves and rhizomes	53, 98, 79, 93, 107, 121, 136	1034	(A)	136	green, warm, floral, herbal sweet		1,2,3,4,5,6,8,9
γ- terpinene	Oils from fresh and dried rhizomes	43, 77, 93, 121, 136	1065	(A)	136.23	terpenic, sweet, citrus, lime-like, oily green with a tropical fruity nuance		1,2,7,9
epoxy myrcene	Fresh rhizomes	41, 59, 71, 79, 93	1094	(B5)	152.2	earthy, musky and fruity		

linalool	Oils from fresh and dried rhizomes	43, 55, 69, 71, 80, 93, 121	1101	(A)	154	citrus, orange, lemon, floral, waxy aldehydic woody		1,2,6,7,9
allo ocimene	Oils from leaves, fresh and dried rhizomes	79, 91, 105, 121, 16	1131	(A)	136.23	citrus, terpenic, tropical, spicy nutty		1
terpinen-4-ol	Oils from leaves, fresh and dried rhizomes	43, 55, 71, 93, 111, 136, 153	1188	(A)	154	orange, floral, fermented, cheesy		2,3,7
perilla alcohol	Oils from leaves, fresh and dried rhizomes	41, 55, 67, 79, 93, 121	1309	(A)	152.23	woody, spicy, dry, bitter		2,3,4

tricyclene	Oil from fresh leaves and rhizomes	79, 93, 105, 121, 136	921	(A)	136.23	strong odour		1
p- cymene	Oil from fresh leaves and rhizomes	91, 119, 134	1032	(A)	134.22	terpenic, citrusy orange fresh		1,2,3,4,5,8,10
eucalyptol	Oils from fresh and dried rhizomes	43, 55, 71, 81, 93, 108, 139, 154	1039	(A)	154.2	strong, fresh, minty, camphoraceous (like eucalyptus), balsamic (like lavender oil)		1,2,3,4,6,7,8,9,10
β-(E)-ocimene	Oils from fresh and dried rhizomes	41, 79, 93, 105, 121, 136	1050	(A)	136	sweet, herby		1,2,4,5,7,8
borneol	Oils from leaves, fresh and dried rhizomes	95, 110, 121, 139	1178	(A)	154	earthy, woody, peppery, musty, lime, thyme like		1,2,4,7,10

trans- pinocarveol	Oils from fresh and dried rhizomes	41, 55, 70, 83, 92, 109, 134	1152	(B6)	152	Flowery		8
pinocarvone	Oils from fresh and dried rhizomes	41, 53, 81, 108, 135, 150	1175	(A)	150.22	balsamic, woody, earthy, minty		

[a] Linear retention index on a HP-5MS column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited– [B1 - (Ciążyńska–Halarewicz and Kowalska, 2003),,B2 -(Venskutonis et al., 2002–,B3 - (Angioni et al., 2006), B4 - (Chung et al., 2003), B5 - (Adams and Nguyen, 2005), B6 - (Chalchat and Özcan, 2005), B7 - (Rodrigues et al., 2013)]; [c] Other reported references [(1-(Padalia et al., 2013), 2-(Srivastava et al., 2001), 3-(Singh et al., 2003), 4-(Tamta et al., 2016), 5-(Choudhury et al., 1996b), 6-(Dutt and Tayal, 1941), 7-(Mustafa et al., 2005), 8-(George et al., 2015), 9-(Rao et al., 1989), 10-(Prakas*h* et al., 2005), 11-(Jatoi et al., 2007)].

Table 3 – Relative abundance of volatile compounds identified in the headspace of mango ginger using SPME GCMS at three different storage temperatures (T1 (8 °C); T2 (15 °C); C (Control; 22 °C) at three different durations (D1 (day 1); D15 (day 15); D29 (day 29).

		Mean relative abundance (AU) [j]											
		T1 [a]			T2 [b]			C [c]			P value [k]		
Code	Compound	D1 [d]	D15 [e]	D29 [f]	D1	D15	D29	D1	D15	D29	T [g]	D [h]	TxD [i]
	Alcohols												
A1	1 - pentanol	0.01±0.04 ab	0.05±0.05 a	0.082±0.032 ab	0.12±0.02 ab	0.09±0.04 ab	0.11±0.03 ab	0.18±0.13 b	0.11±0.03 ab	0.11±0.04 ab	*	*	ns
A2	3-hexen-1-ol Z	0.22±0.29 a	0.59±0.25 ab	0.02±0.05 a	0.20±0.308 a	0.29±0.451 a	0.03±0.06 a	0.40±0.53 ab	1.22±1.09 b	0±0 a	ns	**	ns
A3	hexanol	0.39±0.08 ab	0.32±0.07 ab	0.29±0.05 a	0.40±0.08 ab	0.34±0.23 ab	0.40±0.05 ab	0.57±0.27 ab	0.64±0.33 b	0.41±0.06 ab	**	ns	ns
A4	2 heptanol	0.23±0.10	0.44±0.44	0.43±0.26	0.38±0.14	1.11±0.63	1.00±1.04	0.85±0.45	1.34±1.54	0.30±0.21	ns	ns	ns
A5	2-ethyl hexanol	0.91±0.17 abc	0.49±0.26 a	0.78±0.18 abc	1.00±0.17 abc	0.88±0.42 abc	1.13±0.19 bc	1.29±0.45 c	0.67±0.38 ab	0.99±0.16 abc	**	**	ns
A6	2-nonanol	16±7.94	12±4.56	19±8.79	12±5.52	20±10.19	16±7.11	14±6.84	12±4.34	13±3.80	ns	ns	ns
	Total	**17**	**14**	**20**	**14**	**23**	**19**	**17**	**16**	**14**			
	Aldehydes												
AL1	hexanal	0.74±0.42	0.92±0.25	0.39±0.15	0.64±0.26 a	1.25±0.96 ab	0.58±0.10 a	0.69±0.25a	1.97±0.99b	0.49±0.16 a	ns	***	ns
AL2	2-ethylhexanal	0.21±0.29 a	0.35±0.15 ab	nd	0.21±0.33 a	0.18±0.25 a	0.03±0.03 a	0.28±0.14 ab	0.67±0.47 b	0.01±0.03 a	ns	***	ns
AL3	benzaldehyde	0.58±0.16	0.72±0.22	0.76±0.25	0.53±0.14	0.96±0.46	0.93±0.17	0.51±0.22	0.65±0.21	0.65±0.21	*	**	ns
	Total	**2.0**	**2.0**	**1.0**	**1.0**	**2.0**	**2.0**	**1.0**	**3.0**	**1.0**			
	Esters												

E1	methyl butanoate	0.18±0.04	0.16±0.02	0.18±0.09	0.19±0.02	0.19±0.02	0.21±0.02	0.15±0.02	0.16±0.04	0.18±0.02	ns	ns	ns
E2	bornyl acetate	0.37±0.62	nd	nd	0.09±0.15	0.08±0.10	0.12±0.18	0.11±0.26	0.04±0.09	0.41±0.15	ns	ns	*
E3	isopropyl tetradeconate	0.44±0.08 ab	0.44±0.23 ab	1.0±0.33 b	0.11±0.13 a	0.45±0.19 ab	3.0±1.37 c	nd	0.47±0.15 ab	1.0±0.33 b	*	***	**
	Total	**1.0**	**1.0**	**1.0**	**0.4**	**1.0**	**3.0**	**0.3**	**1.0**	**2.0**			
	Ketones												
K1	2 hexanone	0.09±0.04 cd	0.02±0.043 ab	0.01±0.02 a	0.11±0.03 d	0.08±0.06 bcd	0.07±0.04 abcd	0.07±0.04 abcd	0.03±0.02 abc	0.05±0.03 abcd	**	**	ns
K2	2 Heptanone	0.16±0.04	0.18±0.09	0.17±0.05	0.19±0.03	0.37±0.15	0.29±0.21	0.32±0.19	0.64±1.08	0.16±0.04	ns	ns	ns
K3	2 nonanone	20±8	22±13	24±14	15±8	33±14	25±14	17±6	22 ±12	15±6	ns	ns	ns
K4	Decanone 2	1.67±1.14 b	0.08±0.21 ab	1.64±1.68 ab	0.73±0.1 ab	0.86±1.36 ab	nd	0.10±0.25 ab	nd	nd	**	ns	*
K5	2-undecanone	4.44±1.75	4.45±2.62	5.59±3.03	2.68±1.60	5.39±2.83	3.35±2.05	3.10±2	2.99±1.02	2.47±0.91	*	ns	ns
	Total	**26**	**27**	**31**	**19**	**40**	**29**	**21**	**26**	**18**			
	Sesquiterpenes												
S1	β - elemene	0.70±0.85	0.39±0.58	0.70±0.31	0.73±0.90	2.0±4.2	1.0±2.18	0.41±0.44	0.64±0.89	0.49±0.56	ns	ns	ns
S2	E-caryophyllene	121±50	85±40	125±75	58±36	70±38	67±31	60±37	59±24	53±21	**	ns	ns
S3	β -farnesene	3.0±1.59	3.0±2.60	4.0±2.40	2.0±1.17	1.0±0.63	3.0±2.0	2.0±1.71	1.0±1.04	2.0±0.78	**	ns	ns
S4	α-humulene	7.0±2.4 ab	5.0±2.17 ab	9.0±5.53 b	3.0±2.01 a	4.0±2.0 a	5.0±2.21 ab	4.0±2.24 a	3.0±1.11 a	4.0±1.69 a	**	ns	ns

S5	curzerenone	0.46±0.54	0.79±1.64	2.0±2.45	5.0±9.18	7.0±16	4.0±7.0	1.0±1.31	0.58±1.02	1.08±1.82	ns	ns	ns
	Total	**132**	**94**	**141**	**69**	**83**	**80**	**67**	**64**	**60**			
	Terpenes												
T1	α-thujene	2.0±0.53 ab	1.5±0.56 ab	2.0±0.89 b	1.0±0.35 a	1.7±0.54 ab	2.0±0.61 b	1.0±0.38 a	1.0±0.73 ab	2.0±0.15 ab	ns	**	ns
T2	α-pinene	22±8.61 ab	21±9.50 ab	28±12.80 b	12±5.29 a	24. ±9.40 ab	28±8.30 b	10±3.67 a	21±8.20 ab	22±3.45 ab	ns	**	ns
T3	camphene	6.0±2.39 abc	7.0±3.24 abc	10±4.50 c	3.0±1.01 ab	8.0±2.49 bc	8.0±1.91 c	2.0±0.65 a	6.0±2.19 abc	7.0±1.38 abc	*	***	ns
T4	sabinene	5.0±2.34 ab	5.0±2.80 ab	6.0±2.51 ab	4.0±2.22 ab	8.0±3.52 b	6.0±1.69 ab	3.0±0.98 a	6.0±2.41 ab	5.0±0.67 ab	ns	*	ns
T5	β-pinene	179±73 ab	189±83 ab	159±68 ab	117±67 ab	232±104 b	166±43 ab	94±30 a	181±66 ab	128±13 ab	ns	**	ns
T6	myrcene	1061±300a	1235±350 a	923±355 a	852±366 a	1348±561a	939±250a	846±292 a	1029±401ab	725±72a	ns	*	ns
T7	δ-3-carene	1.0±0.65	0.92±0.986	2.0±1.20	1.0±0.57	2.0±1.05	3.0±0.73	2.0±0.41	2.0±1.2	2.0±0.26	ns	**	ns
T8	α-terpinene	2.0±1.0 ab	2.0±1.62 ab	4.0±2.31 ab	1.7±0.83 ab	3.0±1.4 ab	4.0±1.5 b	2.0±0.53 a	3.0±1.853 ab	04.0±0.48 ab	ns	**	ns
T9	limonene	14.±6.0 ab	16±8.0 ab	23±12.5 b	10±5.0 a	19±8.20 ab	21±7.02 ab	9.0±2.80 a	16±9.5 ab	14±2.10 ab	ns	**	ns
T10	γ- terpinene	2.0±0.84 abc	2.0±1.14 a	4.0±2.14 c	2.0±0.70 a	2.0±0.1 abc	4.0±2.0 bc	2.0±0.50 a	2.0±2.0 ab	3.0±0.83 abc	ns	***	ns
T11	epoxy myrcene	19.0±18 a	21±29 ab	13±9.0 a	27±18 ab	53±18 b	24±8.0 ab	23±12 ab	42±24 ab	24±9.0 ab	*	**	ns
T12	linalool	15±6.0	9.0±10	13±7	18±8.0	24±14	16±4.60	18±10	16±7.6	14±4.40	*	ns	ns

T13	allo ocimene	3.0±1.14	2.0±2.0	4.0±2.0	2.0±1.0	3.0±1.30	3.0±1.15	1.0±1.10	3.0±2.21	3.0±1.12 a	ns	*	ns
T14	terpinen-4-ol	4.0±1.40 b	0.43±1.1 a	4.0±1.73 b	3.0±1.32 ab	4.0±1.34 b	4.0±1.70 b	2.0±2.80 ab	3.0±1.83 ab	5.0±1.37 b	ns	**	**
T15	perilla alcohol	2.0±2.0	3.0±4.0	2.0±1.62	20. ±1.36	5.0±1.67	4.0±2.37	3.0±1.56	5.0±4.21	5.0±1.56	ns	ns	ns
T16	tricyclene	0.26±0.08 ab	0.23±0.09 ab	0.43±0.19 b	0.14±0.04 ab	0.27±0.01 ab	0.38±0.10 ab	0.12±0.03 a	0.39±0.42 ab	0.29±0.04 ab	ns	**	ns
T17	p-cymene	0.93±0.44	1.0±0.72	2.0±0.92	0.73±0.33	4.0±7.38	2.0±0.74	0.75±0.29	1.46±1.19	1.45±0.21	ns	ns	ns
T18	eucalyptol	41.0±17	47.0±22	46±23	32±15.0	55.0±25	52.0±18.0	44±31	57±43	35±7.0	ns	ns	ns
T19	β-(E)-ocimene	238±100	226±98	238±118	152±87	242±141	214±71	146±56	205±141	147±41	ns	ns	ns
T20	borneol	10±3.20	13±7.20	10±5.71	7.0±3.0	12.0±4.0	9.0±3.0	8.0±6.0	10±4.0	9.0±2.50	ns	ns	ns
T21	trans pinocarveol	0.10±0.75	0.75±1.84	0.85±0.57	1.0±0.73	2.0±1.24	1.0±0.35	1.0±0.77	2.0±1.12	1.0±0.38	ns	ns	ns
T22	pinocarvone	0.77±0.52	1.0±1.19	0.94±0.53	0.66±0.34	1.0±0.54	1.0±0.50	0.72±0.48	1.0±0.83	0.10±0.28	ns	*	ns
	Total	**1630**	**1805**	**1498**	**1249**	**2055**	**1514**	**1219**	**1612**	**1155**			

[a] T1 (8 °C); [b] T2 (15 °C); [c] C (Control; 22 °C); [d] D1 (day 1); [e] D15 (day 15); [f] D29 (day 29); [g] Effect of temperature; [h] Effect of storage duration; [i] TXD Temperature X Day interaction; [j] Estimated quantities (mg) collected in the headspace of mango ginger samples containing 1 mL of saturated calcium chloride with 100mg of fresh sample, calculated by comparison with 100 µg/mL propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of six replicate samples are shown; nd, not detected; ns, not significant probability obtained by ANOVA; * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

2.3.2 Biochemical profile is more influenced by duration than temperature

Twenty compounds across the major volatile groups (terpenes, ketones and alcohols) showed a significant impact due to the effect of storage duration (D), when compared to that of either temperature (T) or the combined effect of storage and temperature (T x D). Table 3 shows the changes in quantity (relative abundance values) across the storage duration at three different time points for the three different temperature regimes.

Terpenes (monoterpenes and sesquiterpenes)

Monoterpene compounds showed an overall significant increase in relative abundance by day 15, followed by a decrease by day 29, at 15 °C (Figure 4). These changes were mainly driven by the major terpenes, myrcene, β-(E)-ocimene, β-pinene and eucalyptol, which showed the highest relative abundance within the monoterpene population across all treatments. They also showed highest relative abundance by day 15 across all storage temperature regimes, with a subsequent decrease by day 29 (Table 3 and Figure 4). A significant increase in relative abundance was observed for these three compounds at T2D15 and CD15 treatments followed by a significant decrease by day 29. No significant differences were observed for samples kept at T1 temperatures across the trial. For eucalyptol, the highest relative abundance was recorded at CD15 followed by a significant decrease by day 29. The different temperature treatments did not have any significant effect on β-(E)-ocimene across the trial period. Overall, there was a significant decrease by D29 for T2 (15 °C) and C (22°C) treatments.

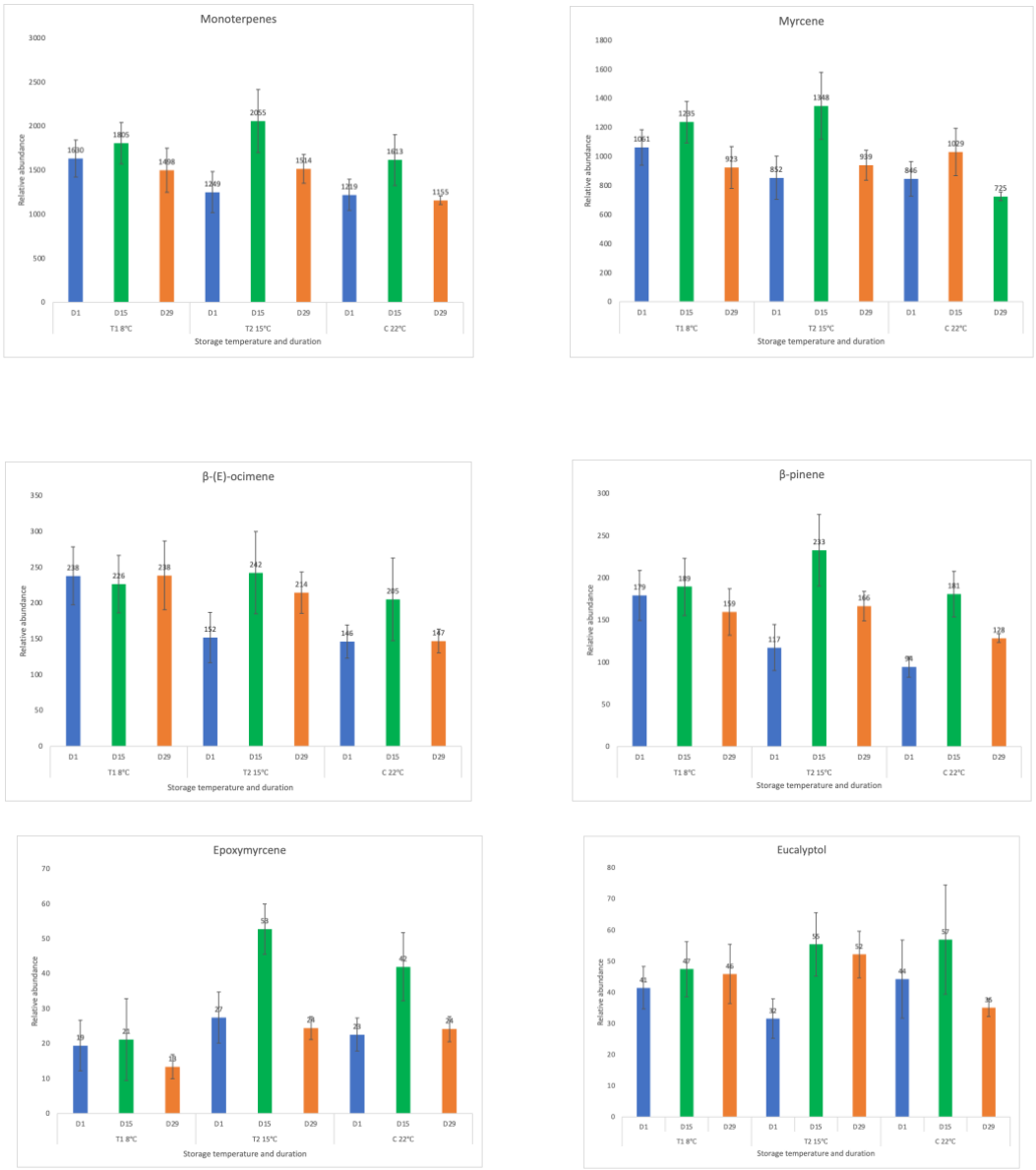


Figure 4 – Changes in relative abundance of monoterpenes, including 5 major monoterpenes in mango ginger across three different storage durations (D1 [day 1]; D15 [day 15]; D29 [day 29]at three different temperature regimes (T1 [8 °C]; T2 [15 °C]; C [Control; 22 °C]). Error bars are calculated using standard error mean values; n=6.

Fifteen monoterpene compounds namely α-thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, car-3-ene, α-terpinene, limonene, γ-terpinene, epoxy myrcene, allo-ocimene, terpinene-4-ol, tricyclene, and pinocarvone showed significant changes in relative abundance due to the impact of storage duration. Although other terpenes showed varying patterns of changes throughout the trial period, the significantly higher relative abundance values of these four compounds were able to skew the behaviour of the overall terpene population to show an initial

increase followed by a decrease across all temperature regimes. The dominance of these terpenes in mango ginger is aligned to other literature (Gholap and Bandyopadhyay, 1984; Rao et al., 1989; Padali*a* et al., 2013).

Only three terpenes were significantly impacted by changes in temperature, namely camphene, epoxy myrcene and linalool. The combined effect of both temperature and storage duration did not have a significant impact on the relative abundance of any terpene compound except terpinen-4-ol.

Monoterpenes produced via the Methyl erythritol (MEP) pathway in the plastids and sesquiterpenes synthesised via the Mevalonic acid (MVA) pathway in cytosol (Dudareva et al., 2004) are known to be produced in response to herbivore attack or as a result of wounding in plants. In the case of volatile terpenes, the induced emissions reflect the elicitation of the MEP pathway, revealing important functions of these compounds as a defence mechanism. This aligns with the initial increase in relative abundance of terpenes between D1 and D15 in the current study, as the rhizome fingers were subjected to separation from the mother rhizome on D0. This separation would have triggered the MEP pathway, thereby increasing the production of terpene compounds.

Another reason for the varying levels of terpene composition throughout the storage duration could be due to pre- and post-harvest handling followed by a lengthy supply chain process which includes exposing the samples at different storage conditions. The rhizome being the storage structure across its storage period undergoes enzymatic reactions which can activate several metabolic reactions resulting in an increase in terpene concentration as a survival mechanism to cope with the stress conditions across the storage time and temperature. The yield and composition of volatile oils have been proven to be affected by environmental factors such as temperature in other studies as well (Staudt and Bertin, 1998; Gershenzon et al., 2000) which aligns with the above results. Changes in volatile terpene constituents arising from changes in storage temperature and duration have also been reported in ginger, when stored at 15 °C for up

to five months and then at 23 °C for three months under high humidity in the dark. The study noted that acyclic oxygenated monoterpenes such as neral and geranial were found to increase to 60 % of the essential oils, whereas that of geraniol and geranyl acetate decreased to an undetectable amount (Sakamura, 1987).

The significant increase in relative abundance of terpenes detected towards day 15 in this study can be attributed as a marker for triggering of senescence processes due to prolonged storage. Many post-harvest studies have confirmed the role of volatile terpene production and its association with senescence (Romagni et al., 2000; Maffei et al., 2001; Chaimovitsh et al., 2010). Other findings have shown that monoterpenes affect biological membranes by damaging their structure and changing their lipid packing density, resulting in increasing ion permeability and perturbs membrane-bound enzyme function (Griffin et al., 2000). These processes increase cell wall permeability and subsequent release of terpene compounds. The subsequent dip in terpene composition by day 29 can be attributed to loss of volatiles due to the senescence-related changes in the cell membrane structures as well as using up of terpene compounds for respiration and other physiological activities during the extended storage period. Volatile terpenes have proven to initiate plant senesence as shown in studies conducted in *Arabidopsis thaliana*, where exposure to α-pinene, β-pinene and camphene were found to result in cell membrane destruction and cell death and ultimately organ senescence (Maffei et al., 2001; Chaimovitsh et al., 2010). An increase in concentration of β-pinene, β-terpinene, fenchyl acetate, camphene and car-3-ene prior to needle abscission was noted in balsam fir (Korankye, 2013), which also suggested the possible role of these volatile terpene compounds in post-harvest senescence or abscission. It has also been reported that volatile terpene compounds also play a major role in plant senescence by keeping the plant healthy and also protecting it against environmental stresses that are known to cause plant death (Vickers et al., 2009).

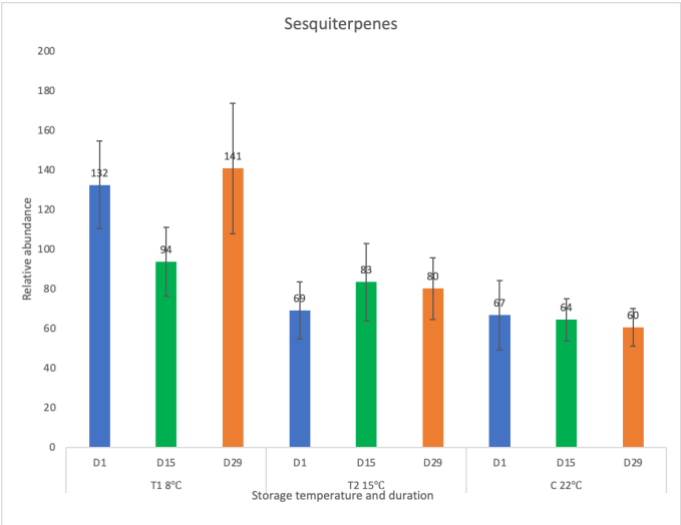


Figure 5 – Changes in relative abundance of sesquiterpenes in mango ginger across three different storage durations at three different temperature regimes (T1 [8 °C]; T2 [15 °C]; C [Control; 22 °C]; D1 [day 1]; D15 [day 15]; D29 [day 29]). Error bars are calculated using standard error mean values; n=6.

Sesquiterpenes were found to be more significantly impacted by temperature, than by duration or T x D interaction The highest relative abundance was recorded for the T1D29 treatment (Figure 5). E-caryophyllene, β-farnesene and α-humulene showed an inverse relationship with temperature, where a significant decrease in relative abundance for all three compounds was noted with increase in temperature (Table 3 and Figure 5). This inverse relationship has been identified in other crops as well such as carrots where a considerable increase of sesquiterpenes have been observed in refrigerated conditions (Kjeldsen et al., 2003).

In addition to terpenes, other group of volatiles known as C6 compounds were also detected in this study. They have been categorised into aldehydes, esters, ketones and alcohols, and are produced via the lipoxygenase (LOX) pathway. Chemically, C6 compounds are mostly saturated or monounsaturated aldehydes, alcohols and esters, and they can have different configurational isomers with different sensory properties (Ruther, 2000).

Esters and Aldehydes

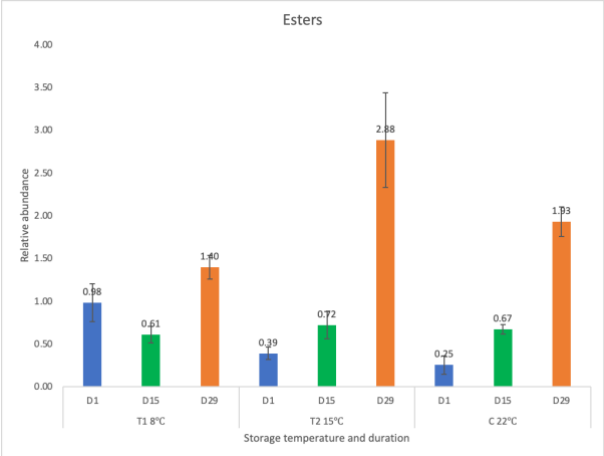


Figure 6 – Changes in relative abundance of esters in mango ginger across three different storage durations at three different temperature regimes (T1 [8 °C]; T2 [15 °C]; C [Control; 22 °C]; D1 [day 1]; D15 [day 15]; D29 [day 29]). Error bars are calculated using standard error mean values; n=6.

Esters as a group showed a significant increase by day 29 across all temperature regimes (Figure 6); among esters, isopropyl tetradeconate was significantly impacted by duration (D) and a combination of temperature and duration (T x D; Table 3). Increase in esters during ripening has been reported in other studies as well, which showed that most types of fruit have the ability to metabolize aldehydes into alcohols, and then into their corresponding esters during ripening as well as storage (Dixon and Hewett, 2000; Pelayo et al., 2003; Alejandro Vazquez-Cruz et al., 2012). Esters, which are mainly derived from the lipoxygenase pathway and amino acid metabolism are associated with the “fruity” attributes of fruit flavour, and their levels typically increase in the later periods of the ripening process during storage. Similar changes in esters due to different storage conditions were also reported in peaches (Cano-Salazar et al., 2013).

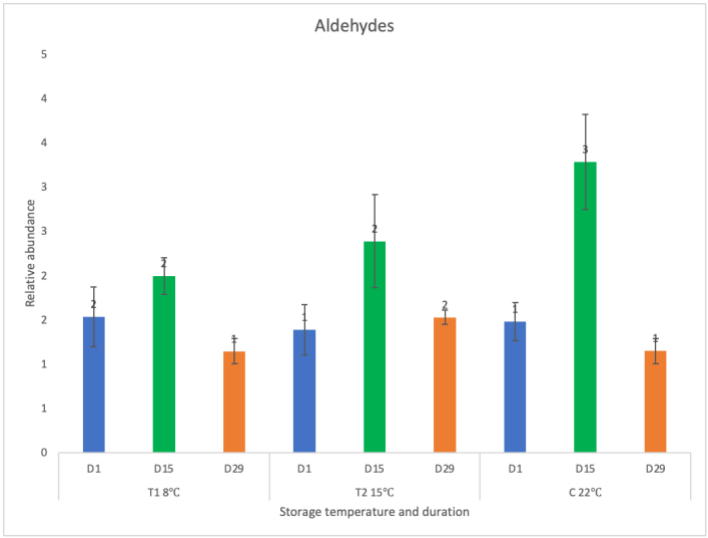


Figure 7 – Changes in relative abundance of aldehydes in mango ginger across three different storage durations at three different temperature regimes (T1 [8 °C]; T2 [15 °C]; C [Control; 22 °C]; D1 [day 1]; D15 [day 15]; D29 [day 29]). Error bars are calculated using standard error mean values; n=6.

Aldehydes showed an opposite trend of decrease in their relative abundance by day 29 followed by an increase at D15 for all the temperature treatments (Figure 7). An increase of esters combined with a decrease in terpenes and aldehydes noted in the current study has also been reported in shelf-life trials of mandarins which developed off flavours after 7 weeks of storage at lower temperatures (Marcilla et al., 2009; Obenland et al., 2009; Tietel et al., 2010; Obenland et al., 2011). This study also reported the development of undesirable flavours in mandarins during storage at different temperature regimes (0 °C, 4 °C, 8 °C and 20 °C); this was found to be associated with the change in concentration of aroma active compounds like esters ultimately impacting the flavour and aroma profile.

Ketones and Alcohols

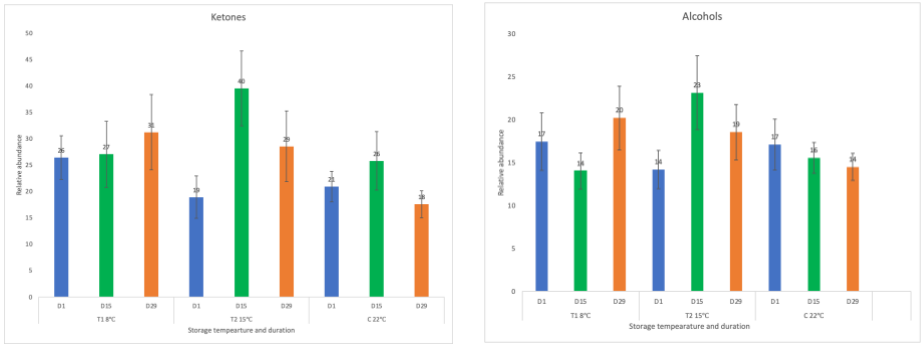


Figure 8– Changes in relative abundance of ketones and alcohols in mango ginger across three different storage durations at three different temperature regimes (T1 [8 °C]; T2 [15 °C]; C [Control; 22 °C]; D1 [day 1]; D15 [day 15]; D29 [day 29]). Error bars are calculated using standard error mean values; n=6.

In ketones there were no significant differences in relative abundance when compared between the temperature treatments, but the lowest relative abundance was recorded in the T2D1 and CD1treatment. Alcohols also showed a similar pattern, but the lowest relative abundance was recorded at T1D15 and T2D1(Figure 8).

C6 volatiles such as esters, ketones, alcohols, aldehydes are usually associated with increased production during ripening in fruits Rodríguezet al., (2013) and account for >50 % of the emissions due to abiotic stress and from damaged plant parts (Loreto et al., 2010). In mango ginger, it can be hypothesized that an increased production of esters and decrease of aldehydes by day 29 can be attributed to the end of the flavour shelf-life period, whereas the high terpene composition during day 15 can be a marker for the start of senescence and later reduction is due to its loss because of the senescence and abiotic stress related changes in the membrane structures.

All agricultural produce under storage respond to their environment as all horticultural commodities undergo natural metabolic processes such as senescence, sprouting, ripening, and may be infected with various groups of microorganisms. These commodities may also undergo various abiotic stress conditions due to changes in temperatures, relative humidity, water, during preharvest and post-harvest conditions, which can trigger biochemical pathways leading to

change in the VOC profile. The biotic or abiotic stresses, as well as other physiochemical changes such as change in colour, total soluble solids, dry matter, oil content are directly correlated with ripening and senescence during storage of horticultural produce (Paul and Pandey, 2014). All these physiochemical changes can lead to aroma and flavour quality fluctuations during storage because of different VOCs produced in response to its storage conditions.

Although some compounds within the other chemical groups such as alcohols, aldehydes, esters and ketones were also significantly impacted by duration, the relative abundance of these compounds were not large enough to create an overall impact to the biochemical profile, when compared to that of terpenes. The significant changes influenced by storage duration was therefore able to create a greater impact on the biochemical profile, when compared to that caused by different storage temperatures.

2.3.3 Sensory Analysis

The sensory profile of fresh mango ginger rhizomes was generated by a trained panel who came to the consensus of 28 terms for the quantitative assessment of samples, grouped into categories such as Appearance, Aroma, Taste / Flavour, Mouthfeel and Aftertaste effects. Table 4 shows the mean panel scores for these attributes.

Table 4 - Mean panel scores for 28 sensory attributes of mango ginger kept under three different storage temperatures (T1 (8 °C); T2 (15 °C); C (Control; 22 °C) at three different durations (D1 (day 1); D15 (day 15); D29 (day 29)

Score [A]												
Code	**Attribute**	**Anchor point**	**T1 (8 °C) D1**	**T1 (8 °C) D15**	**T1 (8 °C) D29**	**T2 (15 °C) D1**	**T2 (15 °C) D15**	**T2 (15 °C) D29**	**C (amb) D1**	**C (amb) D15**	**C (amb) D29**	**P [B]**
	Appearance											
ap01	Shiny	Low to High	27 a	25 ab	12 bc	22 abc	22 abc	16 abc	15 abc	17abc	9.0 c	ns
ap02	Scaley		44	47	49	46	53	42	50	46	47	ns
ap03	Sprouts/Eyes		35	40	40	44	36	39	47	38	34	ns
ap04	Shrivelled		22c	42 b	52 ab	35 bc	46ab	51ab	39 bc	46 ab	61a	***
ap05	Distinct Core	Light to Dark	51	50	56	47	48	52	50	47	45	ns
ap06	Core colour	White to Yellow	61	48	65	55	62	64	57	59	64	ns
	Aroma	Mild to Extreme										
ar01	Green Fresh		38.	31	33	33	24	35	37	27	31	ns
ar02	Floral		15	9	12	12	15	10	11	11	9.0	ns
ar03	Green mango		25	22	21	26	22	23	23	21	22	ns
ar04	Mild turmeric		16	12	11	16	14	9.0	11	8.0	11.0	ns
ar05	Soapy		16ab	17ab	23a	23 ab	27 a	21ab	8.0b	19.0 ab	20 ab	ns
ar06	Piney		39	29	31	30	32	33	40	31	30	ns
	Taste / Flavour	Mild to Extreme										
tf01	Sweet		19	20	24	22	25	23	22	21	15	ns
tf02	Bitter		34	34	32	31	31	28	33	33	36	ns
tf03	Green Fresh		37 ab	28 ab	31 ab	33ab	27 b	32 ab	42 a	30 ab	32 ab	ns

tf04	Mild turmeric		12	9.0	10	16	12	10	15	10	11	ns
tf05	Piney		43ab	28b	38 ab	36ab	32ab	38 ab	47a	37ab	34ab	ns
tf06	Soapy		36	39	40	37	38	33	33	34	35	ns
	Mouth Feel											
mf01	Crunchy	Less to High	60	50	50	49	46	57	60	51	55	ns
mf02	Moist		55a	46ab	42ab	52a	47 ab	43ab	52a	47ab	36b	ns
mf03	Warming		43	38	35	40	35	31	39	40	32	ns
mf04	Fibrous		38b	46 ab	52 a	46 ab	46ab	41ab	46 ab	46ab	51a	ns
	Aftertaste Effects											
af01	Throat Burning	Mild to Extreme	47	45	45	41	42	43	41	44	41	ns
af02	Salivating		28	31	27	24	27	26	25	28	27	ns
af03	Mouth Drying		31	37	32	33	36	31	33	40	37	ns
af04	Soapy		35	32	37	37	36	31	30	30	33	ns
af05	Bitter		33	32	29	31	29	27	34	30	32	ns
af06	Mouth Burning		23	29	32	23	24	25	21	25	27	ns

$_A$: Means are from two replicate samples; differing small letters (a, b, c) represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$). $_B$: Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5 % level; * significant at the 1 % level; ** significant at 0.1 % level. n=11

Although a few studies have previously been done to identify the aroma volatiles in mango ginger, literature related to sensory studies conducted on its organoleptic properties is sparse. To our knowledge, no previous sensory trials have been reported on raw mango ginger rhizomes to determine the impact of different storage and temperature regimes on its flavour and aroma profile.

Instrumental analysis performed for this study showed that myrcene, β-(E)-ocimene and β-pinene were the dominant terpenes within the mango ginger rhizome. Previous flavour profile studies conducted in mango fruits have reported a strong correlation of green mango aroma, floral aroma and leafy mango aroma with terpenes like *cis*-ocimene, α-pinene, and car-3-ene (Ramteke et al., 1981). As these terpenes were also identified in the current study, a similar flavour was expected to be reported from the sensory trials as well. On the contrary, sensory analysis concluded that the overarching flavour throughout the storage duration was an aftertaste resulting from throat burning, mouth drying and salivating effects. Soapiness and pineyness also were found to score higher than green mango and mild turmeric flavours, which contributed to the overall aftertaste by masking all other flavours making it difficult for the panellists to detect them.

The main reason for this divergence from the above study can be attributed to the differences in the terpene composition of mango ginger, when compared to mangoes. GCMS studies conducted in Jerusalem artichoke tubers (Bach et al., 2012) have reported unpleasant sensory attributes due the dominance of terpenes like β-bisabolene and α-pinene. Studies conducted in carrots (Kreutzmann et al., 2008; Fukuda et al., 2013) and mandarins (Pott et al., 2020) also reveal that the accumulation of monoterpenes leads to harsh and bitter flavours with a burning aftertaste, sometimes combined with unpleasant musty notes. Although other chemical groups such as aldehydes, ketones, alcohols and esters which can create favourable aroma and flavour attributes ranging from fresh, fruity, woody, fatty and waxy, were identified in the chemical analysis, the relative abundance of these groups were not high enough to negate the effects of terpenes which dominated the biochemical profile.

It is also important to note that sensory perception is influenced by both volatile and non-volatile compounds, whereas instrumental studies in mango ginger have only focused on volatile compounds. As a result, it is not always possible to associate a linear relationship between chemical composition and sensory results. It may be reasonably assumed that the strong aftertaste sensation is the result of complex interactions between individual components that make up the biochemical profile.

Panellists were also unable to identify any significant differences in aroma and flavour attributes over the trial period. The only attribute that showed a significant difference was Shrivelling; the values of all other attributes were largely consistent and did not appear to be significantly impacted by storage temperature or duration. It was noted that all of the aftertaste attributes scored much higher than other categories; this was also highlighted in individual comments from panellists.

Studies conducted on Pomegranates (Mayuoni-Kirshinbaum et al., 2013) have reported similar findings that prolonged storage under commercial modified atmospheres conditions yielded high preference scores during the first 12 weeks of cold storage at 7 °C, but sensory quality decreased remarkably after 16 and, especially, 20 weeks. Descriptive flavour analyses by a trained sensory panel revealed that the decrease in fruit flavour preference resulted mainly from a significant decrease in typical pomegranate flavour and increases in ‘overripe’ and ‘off-flavour’ odours. Aroma analysis suggested that the decrease in flavour preference most likely resulted from accumulation of ethanol and sesquiterpene volatiles. The study also detected a significant accumulation of monoterpene and sesquiterpene volatiles during this time. Similar findings relating to the effect of storage duration on aroma volatile changes have been reported for other fruit, such as strawberries, mandarins, and apples (Ke et al., 1994; Rudell et al., 2002; Tietel et al., 2011).

Certain challenges were also faced during the sensory trial period which can be attributed to the predominance of aftertaste scores. Due to the exotic nature of the aroma and flavour profile of

mango ginger, samples of mature green mangoes were planned to be provided to the panellists as reference materials. However, owing to the COVID-19 pandemic, it was only possible to procure semi-ripe mangoes. This resulted in a lack of appropriate reference material, which combined with the relative inexperience of the panellists with exotic produce, could have played a part in the sensory results being skewed towards aftertaste attributes.

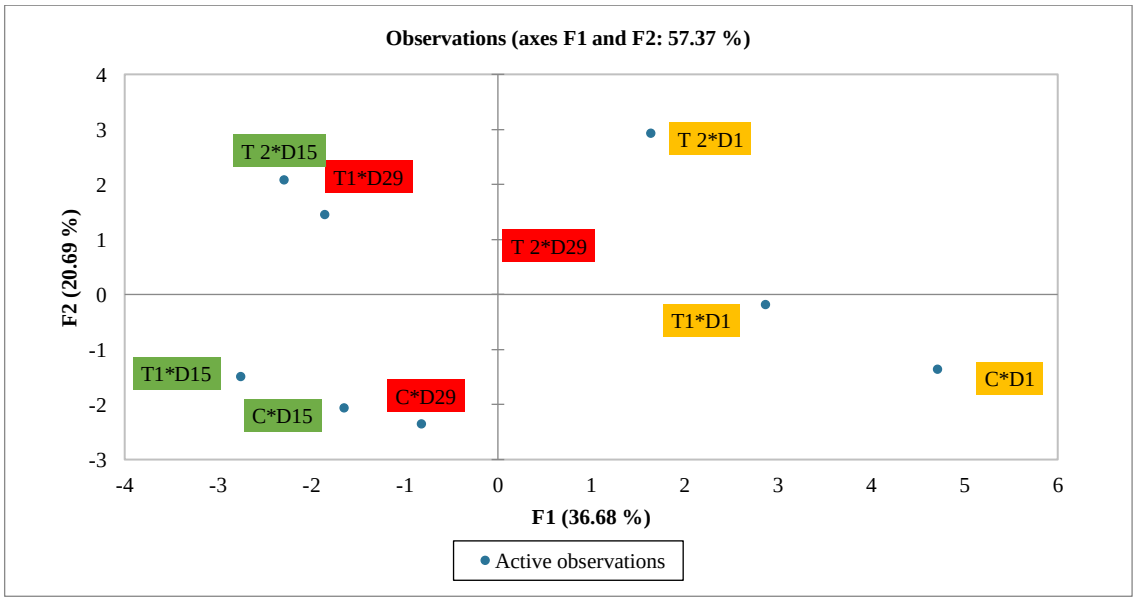
Distinguishing between the different compounds that make up a mixture is often a daunting task for the human brain (Bell et al., 2018) Constituent compounds are often combined by the brain to perceive a different sensation than would have been identified if each of the compounds were sensed individually. All food samples are composed of many different tastes and volatile aromas ("natural mixtures"), and humans vary in their ability to detect, identify, describe, and cognitively "separate" these from each other (Parker et al., 2014). Studies that have reconstituted taste and flavours have illustrated that characteristic "profiles" associated with foods and beverages are a result of complex interactions between volatile components, as well as with non-volatiles (Frank et al., 2011). These could be the reasons behind the sensory panel providing higher scores for aftertaste attributes.

The strong aftertaste could also be due to the combined effects of various volatiles, non-volatiles and phenolic compounds on the trigeminal nerves responsible for sensation of flavour (Bell et al., 2018). As a result, scores for aroma and flavour attributes were relatively low compared to those of appearance attributes. The effect of complex compounds on the trigeminal nerve have been reported in a study about isothiocyanates compounds by (Lawless and Heymann, 2010).Harsh taste sensation is the result of the interaction between such compounds and pain receptors feeding into the trigeminal nerves. Typical examples are mustard, horseradish and rocket which give off a pungent and burning sensation upon consumption (Bell et al., 2018). Similar results were also reported from capsaicin from chilli peppers (McKemy et al., 2002). Many of the compounds found in mango ginger are therefore likely to act synergistically to create distinctive flavours; however, due to the non-availability of food grade myrcene, car-3-ene and β-(E)-ocimene standards, this hypothesis has never been tested.

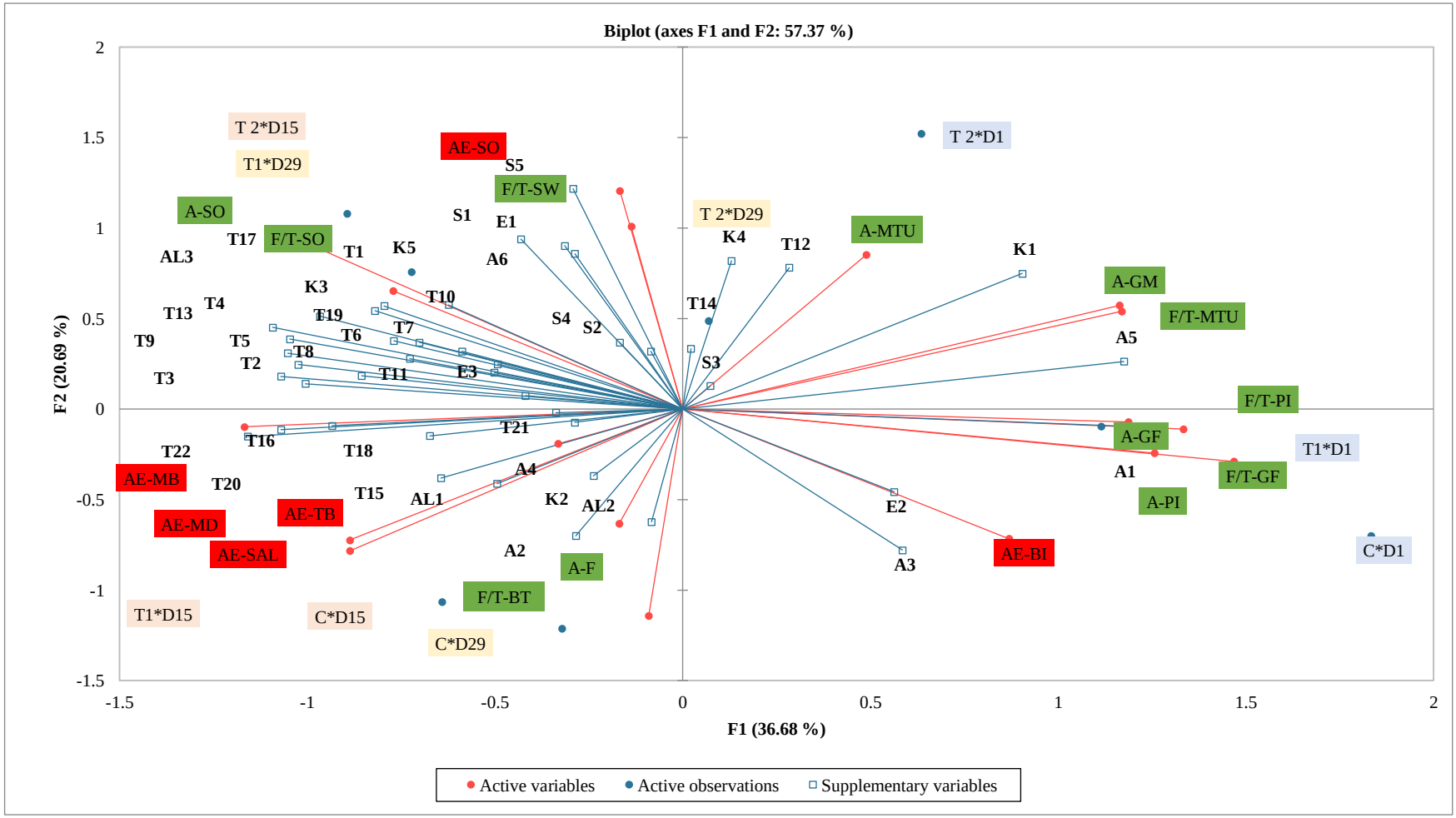
2.3.4 Principal Component Analysis

Principal Component Analysis (PCA) allowed for the visual comparison of the volatile composition of mango ginger to its organoleptic properties by overlapping the HS-SPME and sensory data. The 44 VOCs identified were analysed with the aroma, flavour and aftereffect attributes to get an overview on how variations in sensory quality and chemical composition were influenced by the different storage temperatures and storage time.

(A)



(B)


Biplot (axes F1 and F2: 57.37 %)
F2 (20.69 %)
F1 (36.68 %)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-1.5
-1
-0.5
0
0.5
1
1.5
2
T 2*D15
T1*D29
AE-SO
S5
F/T-SW
T 2*D1
A-SO
S1
E1
T 2*D29
K4
T12
A-MTU
K1
T17
F/T-SO
T1
K5
A6
AL3
K3
T10
T14
A-GM
T4
T13
T19
S4
S2
F/T-MTU
T9
T5
T6
T7
A5
T8
T2
S3
T3
T11
E3
F/T-PI
T21
A-GF
T16
T18
T1*D1
T22
A4
A1
AE-MB
T20
F/T-GF
T15
AL1
K2
AL2
E2
A-PI
AE-MD
AE-TB
AE-SAL
A2
A-F
A3
AE-BI
C*D1
F/T-BT
T1*D15
C*D15
C*D29
Active variables
Active observations
Supplementary variables

(C)

Code	Volatile compound	Code	Volatile compound
Alcohols		**Terpenes**	
A1	pentanol	T1	α-thujene
A2	(Z)-3-hexen-1-ol	T2	α-pinene
A3	hexanol	T3	camphene
A4	2 -heptanol	T4	sabinene
A5	2-ethyl hexanol	T5	β -pinene
A6	2-nonanol	T6	myrcene
Aldehydes		T7	δ-3-carene
AL1	hexanal	T8	α -terpinene
AL2	2-ethylhexanal	T9	limonene
AL3	benzaldehyde	T10	γ- terpinene
Esters		T11	epoxy myrcene
E1	methyl butanoate	T12	linalool
E2	bornyl acetate	T13	allo ocimene
E3	isopropyl tetradeconate	T14	terpinen- 4 -ol
Sesquiterpenes		T15	perilla alcohol
S1	β -elemene	T16	tricyclene
S2	E-caryophyllene	T17	p-cymene
S3	β -farnesene	T18	eucalyptol
S4	α-humulene	T19	β-(E)-ocimene
S5	curzerenone	T20	borneol

		T21	trans pinocarveol
		T22	pinocarvone
Code	**Sensory attribute**	**Code**	**Sensory attribute**
Aroma and Flavour		**Aftereffect**	
A-GF	Green Fresh aroma	AE-TB	Throat burn
AF	Floral aroma	AE-SAL	Salivating
A-GM	Green Mango aroma	AE-MD	Mouth drying
A-SO	Soapy	AE-SO	Soapy
F/T-BT	Bitter taste	AE-MB	Mouth burning
F/T-GF	Green fresh taste		
F/T-MTU	Mild turmeric taste		
F/T-PI	Piney taste		
A-MTU	Mild turmeric aroma		

Figure 9. Principal component analysis of mango ginger samples kept at three different temperatures and showing correlation with volatile compounds. (A) Projection of the samples; (B) Distribution of variables; (C) Compound codes as appearing in plot (B).

The results were visualised by means of a biplot (Figure 9A) showing Principal Component one (F1) and two (F2) which explained 57.37 % of variation in sensory attributes observed. A significantly large proportion of the variation in the aftereffect attributes and volatiles were found in F1 (36.68 %), whereas F2 (20.69 %) mainly explained variation in the other flavour attributes and associated volatile components. It is also recorded from the biplot (Figure 9B) that there is a clear segregation between the storage temperature treatments and duration with respect to the aroma, flavour and after effect related organoleptic properties. On the left-hand side of the biplot (top and bottom quadrant) all the aftereffects taste along with soapiness were observed to be positioned together with strong correlation from day 15 to day 29 across all the treatments. From an aroma perspective, green fresh, green mango, mild turmeric and from a flavour perspective, green fresh and mild turmeric showed a positive correlation on day 1, across all the treatments, mainly for T2 temperature shown on the top right-hand side of the quadrant.

The PCA shows strong correlation for the following aroma attributes, flavour attributes and corresponding volatiles (See Appendix 1 for correlation table). From the correlation table it was recorded that many of the sensory attributes assessed share highly significant correlations between them and also with volatiles from different groups. The main aroma and flavour attributes such green mango aroma (A-GM) and mild turmeric aroma (A-MTU) were strongly correlated ($r = 0.709$, $p<0.05$). Green mango aroma (A-GM) was also correlated with mild turmeric flavour (F-MTU) with ($r = 0.775$, $p<0.05$). The ester volatile 2- hexanone (K1) was positively correlated with A-GM and A-MTU ($r = 0.805$, p 0.01; $r =0.687$, $p<0.05$) as seen in the right-hand corner of the biplot (Figure 9 B and C). These correlations agree with the sensory panel findings wherein mild turmeric aroma was perceived along with green mango aroma.

The three flavour attributes, green fresh (F/T-GF), mild turmeric (F/T-MTU) and piney (F/T-PI) were strongly correlated with the alcohol volatile 1-pentanol (A1) ($r=0.809$, $p<0.01$; $r=0.753$, $p<0.5$; $r=0.787$; $p<0.05$). These flavours were also strongly correlated with the alcohol volatile 2-ethyl-1-hexanol (A5) ($r= 0.715$ $p<0.05$; $r=0.668$, $p=<0.05$; $r=0.688$, $p<0.05$).

The mild turmeric flavour(F/T-MTU) was also found to have strong positive correlation with 2-hexanone (K1) (r=0.711, p<0.05) and an inverse correlation with the aftertaste attributes such as salivating (AE-SAL) (r=-0.748, p<0.05) and mouth burning (AE-MB) (r= -0.717, p<0.05). All these aroma and flavour attributes were found to be strongly correlated between D1 and D29 at T2 (15 °C) treatment conditions as seen in the top right and bottom left quadrants.

Along with aroma and flavour components, aftertaste effects such as throat burning (AE-TB) and salivating (AE-SAL) were found to be positively correlated (r-0.726, p<0.05) across T1D15, CD15 and CD29). In addition, some volatiles like E-caryophyllene (S2) β-farnesene (S3), β-(E)-ocimene (T19) were also found to be strongly associated with AE-TB (r=0.830, p<0.01; r=0.678, p<0.05; r=0.776, p<0.05). AE-SAL showed strong correlation with borneol (T20) (r=0.806, p<0.01); mouth drying (MD) with 3-hexen-1-ol (A2) (r=0.732, p<0.05) and hexanal (AL1) (r=0.743; p 0.05); mouth burning (AE-MB) with camphene (T3) (r=0.743, p<0.05) and limonene (T9) (r=0.699, p<0.05); and soapy taste (AE-SO) with 2-heptanone (K4) (r=0.789, p<0.05).

For salivating (AE-SAL) with T20 mouth drying (MD) and A2-3-hexen-1-ol Z r=0.732, p<0.05; AL1 hexanal r=0.743; p 0.05) mouth burning (AE-MB) with T3 camphene r=0.743, p<0.05) and (T9 limonene-0.699, p<0.05) and soapy after taste (AE-SO) was found to be correlated with K4-2 heptanone r=0.789, p<0.05)

It was also found that the ~~c~~haracteristic flavours of green mango and turmeric flavour were negatively correlated to the aftertaste effects like throat burning, mouth drying and mouth burning. This adds weight to the assumption that an increase in terpenes responsible for the aftertaste are able to mask the desirable flavours. This is also supported by the results from the sensory analysis that only a mild level of green mango aroma and turmeric flavour could be perceived by the panellists. The relative strength of aftertaste attributes compared to other flavours was also highlighted by the higher scores given to them.

Although the terpene compounds identified in this study relate to harsh after taste flavour attributes (Rosenfeld et al., 2002), there are other studies that support a correlation between these

terpenes and raw mango flavour. Monoterpene compounds such car-3-ene, β-(E)-ocimene, β-myrcene were used to describe characteristic mango odour in mango ginger rhizome(Padalia et al., 2013; Varadarajan et al., 2018) Though similar compounds were detected in the current study results found that only a mild green mango aroma was perceived across the different storage temperatures and storage times. But among the storage temperature T2 (15 °C) until 15 days was found to be ideal and correlated with the characteristic green mango flavour. It can be concluded that a temperature for storing mango ginger samples of 15 °C for 2 weeks seems to be appropriate for maintaining its unique green mango and turmeric aroma and flavour.

2.4 Conclusion

This study was able to highlight the impact of storage temperature and duration on the profile of volatiles produced by mango ginger rhizomes. Although the array of chemicals identified behaved in varying patterns to the treatments, there was a clear indication that storage duration had a greater impact than that of storage temperature on the relative abundance of terpene compounds, which accounted for a significant majority of VOCs in the rhizome. Overall, there was a significant decrease of terpene compounds over the storage duration when exposed to T2 (15 °C) and Control (22 °C) temperature regimes.

Results of the sensory analysis also aligned with the chemicals analysis indicating that the fresh mango ginger was dominated by the overpowering taste of mainly terpene compounds. PCA analysis using instrumental and sensory data found that the characteristic green mango aroma and mild turmeric flavour were strongly associated with T2D1 storage conditions (15 °C until 2 weeks). It may therefore be hypothesised that this is the ideal storage condition for freshly harvested mango ginger rhizomes. It is also generally known that the 'flavour-life' of fruit and vegetables is often shorter than their overall 'storage life', as determined by the external visual quality of the produce (Aggarwal et al., 2003; Baldwin, 2007; Kader, 2008).

Sensory analysis also identified that panellists were unable to differentiate changes in green mango flavour and aroma owing to the overpowering aftertaste such as throat burning, mouth drying and mouth burning. This can be attributed to the relative dominance of terpene compounds in the rhizome, which are associated with these flavours. The relative inexperience of sensory panellists with exotic tastes and the lack of appropriate reference material during sensory trials could also be attributed to the higher scores allocated by the panellists to the after-taste effects. Gas Chromatography-Olfactometry (GC-O) could be employed to identify the odour active compounds in mango ginger, which can then be used as reference compounds in future to arrive at more accurate sensory results. This should help to provide a linkage between specific

compounds and aroma in the complex matrices, and also highlight the effect of other compounds that could mask or change the sensory perception of aroma compounds.

It should also be taken into consideration that most members of Zingiberaceae, such as ginger and turmeric, are associated with a strong and sometimes pungent after taste when tasted fresh. Future studies should consider presenting mango ginger samples with different food matrices which can help the panellists to correctly score the various aroma and flavour attributes without any interference of masking effects of certain volatile compound groups.

Instrumental analysis mainly identified volatile compounds within the rhizome in the current study. Future studies should include the analysis of non-volatile compounds as well to obtain a more accurate aroma and flavour profile of mango ginger. These findings will also enable a focus on breeding programmes to develop genotypes with an aroma and flavour profile which can withstand longer storage durations with minimal impact on their organoleptic attributes.

CHAPTER 3

3. Changes in VOCs and sensory properties of fresh mango ginger rhizomes subjected to different drying techniques

3.1 Introduction

Food products which retain most of their original flavour and aroma characteristics even after being subjected to commercial processing techniques are gaining popularity as raw materials in the food industry. Many herbs and spices which withstand post-harvest handling and processing can therefore be seen to complement the main ingredients used in the food, beverage and cosmetic industries (Prusinowska and Smigielski, 2015; Jin et al., 2018). This has led to an increase in scientific studies on herbs and spices to optimise different methods of preservation that help them to withstand the abiotic stress factors imposed during their supply chain cycle (Prusinowska and Smigielski, 2015).

Freshly harvested spices and herbs have a high moisture content; so immediate steps to reduce microbial growth and limit biological deterioration should be adopted soon after harvest. Drying is the oldest food preservation method and mainly aims to reduce moisture content and water activity (the availability of moisture for microbial growth; (Maltini et al., 2003) to restrict microbial proliferation so that shelf-life is prolonged, packaging costs are minimised and shipping weight is optimised (Doymaz, 2005; Hamrouni-Sellami et al., 2013). As spices are mostly preferred in their powdered form, this requires the harvested product to be dried prior to being powdered (Borah et al, 2015). Thermal drying is the most common and cost-effective post-harvest technique used to reduce the perishability of freshly harvested produce.

Although a number of conventional drying techniques have been developed over time, such as solar (Özcan et al., 2005), hot-air (Demiray and Tulek, 2014), freeze (Gümüşay et al., 2015) and microwave drying (Arslan et al., 2010), it is important to find the optimal drying conditions and duration for each of product, which mostly depends on the physiology and biochemical profile

of the harvested plant material. The volatile nature of essential oils in spices and herbs make them extremely heat-labile, so an efficient and optimum drying technique and temperature should be used to obtain high quality premium products (Prusinowska and Smigielski, 2015). Reactions such as autoxidation of fats, fibre crystallization, protein denaturation, loss of essential oils and vitamins also take place during drying. Along with microbial safety, importance should also be given for retaining the aroma, flavour, colour, sensory attributes, chemical and biological properties, nutritional value, and appearance of the harvested product.

Loss of volatile constituents in herbs and spices mainly depends on drying parameters and biological characteristics of the plants. Thyme and sage are good exemplars of the impact of processing as evidenced by the work of Venskutonis (1997) who subjected these herbs to oven drying and freeze drying and analysed the effect on aroma constituents. The volatile constituents of these herbs were isolated by dynamic headspace and simultaneous distillation-extraction methods and analysed by capillary gas chromatography coupled with gas chromatography-mass spectrometry (GCMS). A significant reduction in the number of extracted volatiles was found only in the case of drying at 60 °C, mainly as a result of the loss of non-oxygenated monoterpenes. It was also found that the changes in the concentrations of volatiles in head space differed significantly for thyme and sage and it was dependent on the method and the drying temperature. The study concluded that the extensive release of volatiles at 60 °C was the result of the biological structure of the oil gland trichomes of these plants being strongly affected by high temperature.

Studies conducted in mango ginger have demonstrated its therapeutic potential, owing to the rich chemical constituents in the rhizome which possess antimicrobial and antioxidant properties (Policegoudra et al., 2011; Sinha 2003; Voravuthikunchai, 2007). But there is little information on the commercial utilisation of these properties, as a result of which, mango ginger has become as an untapped and underutilised spice from the ginger family (Policegoudra et al., 2011). Despite its therapeutic potential, unique flavour, and aroma properties there is not much literature available on the use of this spice in the food and flavour industries. The only literature available regarding the use of mango ginger as a value-added product is the use of dried powder in making

a nutritionally enriched soup stick (Crassina and Sudha, 2015), which looked at replacement of wheat flour with mango ginger powder and the changes in the nutritional characteristics of the soup stick.

The current study therefore focusses on the effect of different drying techniques on the volatile aroma profile of mango ginger as a pioneer attempt to examine the possibilities of utilisation and exploitation of mango ginger as a value-added product in the food and flavour industries. Tray drying using hot air at 50 °C and freeze drying at -70°C were the two techniques used in this study. The study also looked at three months shelf-life quality of the powders to identify the optimal duration for retention of the key flavour components and sensory properties of the dried product. In addition, this study also characterises the physical properties of sample powders.

3.2 Materials and Methods

3.2.1 Preparation of samples

Experiments were carried out to determine the impact of different drying techniques on fresh mango ginger rhizomes; the first one relying on Instrumental Analysis and the second one on Sensory Analysis. Twelve bags of mango ginger rhizomes were purchased from the Western International wholesale market in Southall on the day it arrived in the market. Each perforated plastic bag contained 5 kg of fresh mango ginger and as per the labels on the bags, the plants were grown in Mumbai, India. They were then transported under ambient conditions and stored in the pilot plant at the University of Reading at 8 °C until further sorting for different drying treatments (hot air drying at 50 °C) and freeze drying at -70 °C) for three months under dark conditions.

Ten rhizomes were taken from the bags and sliced in 2 mm thickness to determine the moisture content of fresh rhizomes. 100g of slices were kept in an aluminium foil dish and subjected to oven baking at 105 °C overnight. The samples were reduced to powder form by this time. The moisture content was calculated using the following formula:

Initial wet weight - Final Dry weight/ wet weight X 100

This treatment was replicated 5 times and the average reading showed that fresh rhizomes had a moisture content of 88 to 92 %.

The remaining rhizomes were divided into three sets; the first set was split equally for hot air drying and freeze-drying treatments. An intake analysis was then performed to remove rhizomes with undesirable characteristics (e.g., those that were undersized, those with physical damage, signs of pest and disease attack and brown spots as a result of chilling injury). The remaining were washed to remove surface adhering dust and dirt, dried skin, and soil. They were dried off with paper towels and then peeled and sliced to 3 to 5 mm thickness manually, using a stainless-steel slicer. The samples were then subjected to the respective drying techniques. The same

procedure was repeated for the remaining two sets of samples. The first set of samples was labelled as Month 1 (M1), the second set as Month 2 (M2) and third set as Month 3 (M3). All the dried samples were powdered and stored under ambient conditions (22 °C) for the duration specified by their labels. Ten replications per sample set (five grams in weight) were selected for instrumental and physical attributes analysis at the end of each month.

3.2.2 Hot air drying (HAD)

A laboratory model hot air convective tray dryer (APEX-ALC-TYPE48AE;240 VOLT) was used to dry the sliced rhizomes (3 – 5 mm thickness). The dryer consisted of a blower, temperature controller-cum indicator unit, and three metal trays. The three metal trays could fit the top middle and lower position inside the drying chamber. Separate On/Off switches for heater and airflow unit were available. The blower threw air over the electric heater, which heated the air and made it move upwards at a rate of 2 m/s in the drying chamber. During its upward motion, the hot air picked up moisture from the wet slices and left the drying chamber through the exhaust.

The sliced rhizomes were spread in a single layer on the drying trays. Each tray could accommodate 3.75 kg of sample. The samples were dried for 12 hours, initially at three different temperatures: 50, 55 and 60 °C to determine the optimum temperature regime. As the latter two temperatures resulted in excessive browning and shrinkage, 50°C was used for hot air drying. The moisture content of the samples was recorded every two hours from all the three trays until completion of experiment. The final moisture level was recorded as 6 %.

3.2.3 Freeze Drying (FD)

Fifty grams each of sliced rhizome samples were spread on fifty aluminium trays. As the samples for freeze drying required a preconditioning treatment, they were subjected to blast freezing at -80°C. Subsequently, they were dehydrated in a laboratory freeze dryer (Edwards, model L4KR, Crawley, England) at -70 °C at a pressure of 5 x 10^3 mbar l/s for 5 days. FD samples had a final moisture content of 4 % - 5.5 %.

3.2.4 Processing, Packing and Storing

The dried samples were collected and stored in airtight containers. They were then ground using a 150 W home spice grinder for 10-20 seconds to produce a powder of uniform size. The grinder was switched off after every 20 seconds to prevent heating of the motor and the container to reduce any heat impact on the volatile compounds of the powder samples. Powders were then sieved through a mesh of 600 µ size. They were then packed in airtight food grade containers. The samples from each powder type were allocated for monthly sensory, and other instrumental analysis. They were stored in a dark place under ambient conditions (temperature controlled at 22 °C) for up to 3 months.

3.2.5 Evaluation of changes by Instrumental Analysis in the volatile aroma profile of fresh mango ginger subjected to hot air drying and freeze drying

Dried samples were analysed for their volatile profile by making tea from the powdered samples. 2 g of powder from each of the HAD and FD samples were brewed with 100mL boiling water for 5 minutes and capped tightly. After brewing for 5 minutes, 3 mL of the tea was added to SPME vials. 1 mL of a saturated solution of calcium chloride and an internal standard of 10 µL (100 ppm) Propyl propionate was added to each 3mL sample. Automated Headspace Solid Phase Microextraction method (HS-SPME) was used as described by Morales-Soto et al.(2015) for the identification of the volatile aroma compounds. The same procedure was repeated for set 2 samples at end of second month and third set of samples at the end of third month.

3.2.5.1 Solid Phase Microextraction (SPME) followed by GCMS

The powdered samples were brewed for 5 min, and 3 mL was selected for further analysis. A saturated solution of calcium chloride (1 mL) was added to the samples to prevent unintended release of volatile compounds due to enzymatic reactions. Analysis was carried out by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA, USA). The SPME fibre

stationary phase was composed of 75 μm divinylbenzene/Carboxen™ on polydimethylsiloxane, Supelco (Bellefonte, PA, USA). Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C.

After extraction, the SPME device was inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column HP-5MS (30 m × 250 μm × 0.25 μm thickness) (Agilent, Santa Clara, CA, USA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C and 6 min at 250 °C isothermal. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature of the injector, interface and detector was 250 °C and the sample injection mode was spitless. Mass spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 m/z and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system. Ten replicates were prepared for each sample totalling twenty samples for each drying treatment. An internal standard of 10μL (100ppm) Propyl propionate was added into each sample.

For data processing, the spectrum from data was controlled and stored by the HP G1034C Chemstation system. VOCs were identified by comparison of each mass authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading) with that of the National Institute of Standards and Technology (NIST) databases, (NIST/EPA/NIH Mass Spectral database, 2011), spectra published in other literature and confirmed in many cases by comparison of their retention indices as shown in ADAMS library. To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound, using the retention times of a homologous series of C6–C25 n-alkanes and by comparing the LRI with those of authentic compounds.

In order to ascertain the quality of the dried product, samples were taken every thirty days and analysed for their flavour volatiles, organoleptic properties and functional characteristics such as

bulk density, water activity, water solubility, absorption, colour change and moisture content until the end of the 3-month storage period. An understanding of these properties is essential in designing processing techniques and predicting the quality of the dried product. They also play a huge part in developing new products with just the required desirable properties of for improving existing products (Krokida et al., 2001).

Bulk density (g/mL)

The bulk density of the powders was determined by gently adding HAD and FD powders into an empty 100mL graduated cylinder. The bulk density was calculated by dividing the mass of powder by the volume occupied in the cylinder. The final value was calculated by using the formula: Mass/Volume.

Water absorption index (WAI) and Water solubility index (WSI)

WAI/WSI were determined using the method explained by (Kaur et al., 2014) with slight modifications. 2 g of sample was suspended in 20 mL distilled water. The suspension was stirred for 30 min followed by centrifugation at 8000 rpm for 30 min. The supernatant and sediment were separated, weighed, and dried. The WAI and WSI were estimated by the following given equation:

WAI (g) = weight of the wet sediment/weight of the dried sediment

WSI (%) = weight of solid in supernatant/weight of the dry sample x 100

Colour change (ΔE) of the product across the shelf-life period

The powdered samples were subjected to colorimetric analysis across the 3-month shelf-life period. The readings were recorded using Hunter Lab Scan II Spectro colorimeter (Hunter Lab, Reston, VA). The instrument, equipped with a D65 illuminant and 2-degree observer optical position, was standardized using a standard white plate (No. LS-13685, X) 79.8, Y 84.67, Z)

91.23). The instrument was calibrated and the samples for analysis were transferred to the container provided for measuring the colour changes across the storage period.

Three parameters for the dried samples, $L*$ (lightness), $a*$ (redness), and $b*$ (yellowness), were used to study the changes in colour. $L*$ represents the lightness or darkness of the sample on the scale of 0–100 where white =100 and dark = 0. Hunter $a*$ represents redness (+) or greenness (−). Hunter $b*$ represents yellowness (+) or blueness (−). The colour of HAD and FD was compared to that of a commercial fresh mango ginger rhizome, which has a moisture content of 92 %.

Fresh mango ginger rhizomes at different stages of physiological maturity, i.e., young rhizomes which did not have a distinct core, older ones which have a distinct yellow core, and over-mature ones which have a dark yellow core colour were selected as standard to compare with powders for the change in colour across its shelf-life. The colour difference was calculated using the formula:

$$\Delta E = \sqrt{(\Delta L*)2 + (\Delta a*)2 + (\Delta b*)2}$$

$$\Delta L* = L* - L*o,\ \Delta a* = a* - a*o,\ \Delta b* = b* - b*o$$

ΔE is the total colour difference across the storage duration; $L*o$, $a*o$, and $b*o$ are the colour parameters of fresh samples used as the reference and L*, a* and b* are the corresponding values for the dried powder (Phoungchandang and Saentaweesuk, 2011).

Final moisture content (%)

Ten replicates for each powder type were analysed for their final moisture content using a moisture meter (Sartorius MA 150). The moisture content of HAD and FD powders was measured via an HB 42-S halogen moisture analyser (Mettler Toledo, UK) at 130 °C. 3 g of powder was placed in the sample pan. The empty sample pan handler and the heating module were closed. The drying time ranged from 2 to 5 minutes for each sample.

Water activity (a_w)

The water activity of the 0.5 g of powder was determined by a Hygrolab C1 water activity meter (E-M-HyLabC1-V1_11).

3.2.6 Evaluation of changes by Sensory Analysis in the volatile aroma profile of fresh mango ginger subjected to hot air drying and freeze drying

Samples were presented in a random, coded fashion over the course of 45-minute sessions. Panellists were asked to evaluate the HAD and FD powders for its aroma and flavour. Three training sessions prior to the actual sensory scoring session were conducted to familiarise the panel and develop vocabulary for the for aroma, flavour, colour and texture of the processed mango ginger powders. Five g from each of the processed samples were packed in small food grade containers and distributed to the panellists. Two sets were provided from each of the HAD and FD samples. The first set was for scoring aroma, colour, and texture attributes of the powder; the second set was for scoring the aroma, colour and flavour attributes of tea made from the powder which was prepared from two g of samples subjected to brewing for 5 minutes with 100mL boiling water. Brewing helped the powder samples release the aroma compounds. The tea thus prepared was assessed for its aroma, flavour, and colour components to produce as many descriptive terms as seemed appropriate (Table 5).

Reference standards such as fresh mango ginger rhizomes were provided to compare between the aroma and flavour of dried powders and fresh samples. The reference samples used where appropriate to ensure agreement of the descriptive terms chosen. Once a consensus set of descriptors for various attributes was established, a formal sensory assessment was conducted.

Table 5 – Lexicon developed by sensory panellists for HAD and FD mango ginger samples (n=11).

Category	Attribute	Anchor points	Agreed definitions
Aroma of powder	Fresh mango ginger	Mild to Extreme	Emitting the smell of freshly cut raw mango ginger rhizome
	Gingery		Emitting the smell of fresh ginger
	Turmeric		Emitting the smell of earthy fresh turmeric
	Musty		Earthy, damp odour
	Piney		Resembling the flavour of a freshly crushed pine leaf
	Milky		Emitting a creamy smell
	Rubbery		Emitting the smell of rubber
Colour and texture of powder	Mustard yellow	Mustard Yellow to Pale Yellow	Colour of mustard yellow powder
	Grainy	Grainy to Floury	Coarse to touch when felt in between thumb and index finger
	Floury		Soft and light when felt in between thumb and index finger
Aroma of Tea	Boiled Potatoes	Mild to Extreme	Emitting starchy smell
	Fresh mango ginger		Emitting the smell of freshly cut raw mango ginger rhizome
	Citrusy		Resembling the flavour of citrusy fruits like orange and lemon
	Gingery		Emitting the smell of fresh ginger
Flavour of Tea	Bitter	Mild to Extreme	Unpleasant, sharp or pungent taste on the tongue
	Fresh mango ginger		Emitting the smell of freshly cut raw mango ginger rhizome
	Fresh wood		Emitting the smell of fresh cut wood
	Earthy		Emitting mushroom like smell
	Citrusy		Resembling the flavour of citrusy fruits like orange and lemon
	Sweet		Pleasant taste associated with sugary food
	Off Flavours		Unacceptable taste and aroma
Mouthfeel and Aftertaste	Throat Drying	Mild to Extreme	The feeling of a dry throat
	Mouth Burning		An unpleasant, sharp or pungent taste on the mouth
	Mouth Drying		The feeling of a dry mouth
	Salivating		Excessive saliva production in the mouth as a result of bitterness; also associated with sharp ‘piney’ness
	Throat Burning		Sensation of throat ‘catching at its back’ due to heat sensation

Quantitative sensory measurement took place from home-based computer screens which were connected to the software used for sensory data acquisition, Compusense (Compusense Inc., Guelph, Ontario, Canada). The samples presented to the panellists included a mixture of samples in powder form and powder in teabags in randomised order. There were two replicates from each sample set. The strength of each attribute for each sample was recorded by the assessors on an unstructured line scale (10 cm, scaled 0-100). This led to an agreed profile comprising seven aroma terms, two each for colour and texture for powder, two attributes for colour, four aroma terms, seven flavour / taste terms, and five mouthfeel-aftertaste terms for tea organoleptic attributes.

3.2.7 Statistical Analysis

The relative abundance was calculated from the data collected by SPME GCMS analysis. Quantitative data for each compound identified in the SPME GCMS analysis were analysed by both one-way and two-way analysis of variance (ANOVA; Table 3) and principal component analysis (PCA; Figure 5) using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine the sample means that differed significantly ($p < 0.05$) between drying technique and storage time of the powdered samples.

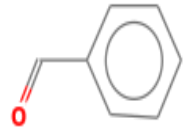
Each sample was presented and assessed twice for sensory analysis by each of the 11 panellists and averaged. SENPAQ version 3.2 (Qi Statistics, Reading, UK) was used to carry out ANOVA (Table 4) and PCA (Figure 5) of sensory panel data. The means for the sensory data were taken over assessors and correlated with the means from instrumental data via principal component analysis (PCA) using XLSTAT.

3.3 Results and Discussion

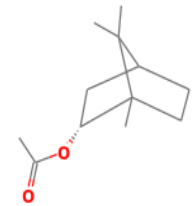
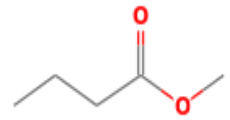
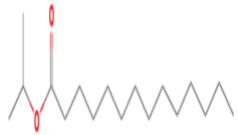
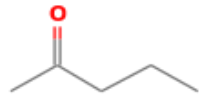
3.3.1 Volatile Composition

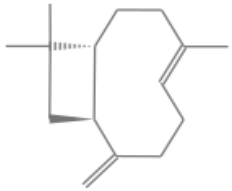
A total of 50 compounds were identified from both tray and FD samples (Table 6). Terpenes were the dominant volatile compounds in both the samples; a total of 28 terpene compounds were identified. Relative abundance value of myrcene (301.246) made it stand out as the major compound, followed by β-pinene (18.553), eucalyptol (16.886), contributing to the volatile aroma profile.

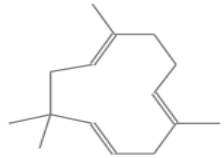
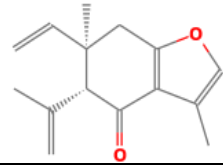
Table 6 – Volatile compounds identified from HAD (50 °C)and FD (-70 °C) mango ginger samples

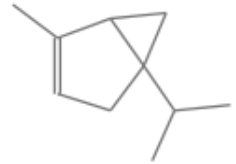
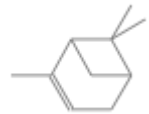
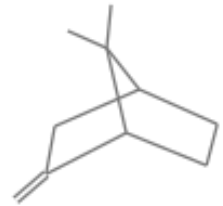
Compounds	Material/Source	Active ions	LRI expt [a]	ID[b]	Molecular weight (g/mol)	Organoleptic properties	Molecular Structure
hexanal	Fresh and dried rhizomes	27,41,44,56,57,82	802	(A)	100.15	dry green, herbal, aldehydic, citrusy	
heptanal	HAD & FD powder	29,41,44,55,57,70, 81,96	903	(A)	114.18	green grassy, fruity, strong, harsh	
benzaldehyde	Fresh and dried rhizomes	51,77,105,106	966	(A)	106.12	fruity, woody, sweet, spicy	
octanal	HAD & FD powder	29,43,57,71,85,142	1003	(B1)	128.21	aldehydic, orange peel , citrusy	
dodecanal	HAD & FD powder	41,57,67,82,96,110	1409	(A)	184.31	soapy, waxy, citrusy	

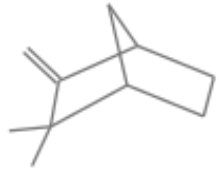
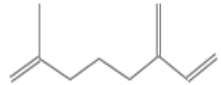
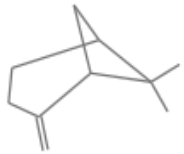
decane	HAD & FD powder	29,43,57,71,85,142	999	(A)	142.28	Odourless	
undecane	HAD & FD powder	43,57,71,86,	1098	(A)	156.3	Odourless	
dodecene	HAD & FD powder	41,55,83,97,168	1187	(A)	168	Odourless	
tetradecene	HAD & FD powder	41,68,154	1388	(A)	196.37	Odourless	

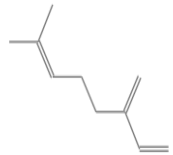
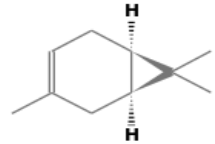
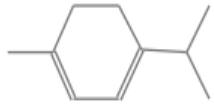
bornyl acetate	Fresh and dried rhizomes	43,95,121,136	1295	(A)	196	balsamic, camphoreous, gin like, woody, soapy	
methyl butanoate	Fresh and dried rhizomes	43,55,71,74,87	721	(A)	102.13	fruity, pungent, acidic, creamy	
methyl dodecanoate	HAD & FD powder	74,87,214	1526	(B7)	214.34	fermented, fatty	
isopropyl tetradecanoate	Fresh and dried rhizomes	43,60,102,228	1824	(A)	270.5	Odourless	
2-pentanone	HAD & FD powder	43,55,70,85	690	(A)	86.13	fruity, pungent, acidic, creamy	

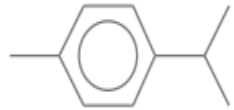
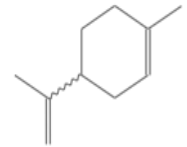
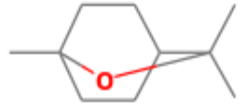
2-nonanone	Fresh and dried rhizomes	43,58,71,142	1091	(A)	142.23	fruity, cheesy, buttery, herbal	
2-undecanone	Oil from fresh leaves, rhizomes, HAD & FD powder	43,57,71	1292	(A)	170.3	fruity, waxy, creamy	
E-caryophyllene	Oil from fresh leaves, rhizomes, HAD & FD powder	96,121,136,147	1467	(A)	204	spicy, woody, pepperlike, citrusy	

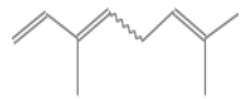
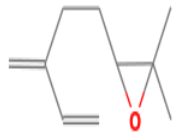
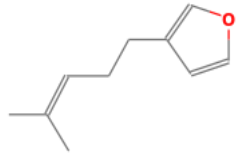
α-humulene	Oil from fresh leaves, rhizomes, HAD & FD powder	80,93,121	1477	(A)	204	woody, spicy, clove, citrusy	
curzerenone	Fresh and dried rhizomes	94,122,230	1626	(A)	230	Earthy, turmeric	
tricyclene	Oil from fresh leaves, rhizomes, HAD & FD powder	41,79,93,121,136	929	(A)	136.23	strong odour	

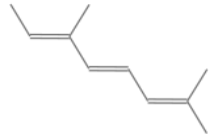
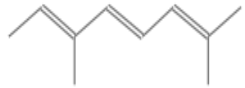
α-thujene	Fresh and dried rhizomes	77,91,93136	932	(A)	136.23	woody, green, herbal	
α-pinene	Fresh and dried rhizomes	27,77,91,93,105,121,136	940	(A)	136.23	herbal, woody, piney, terpenic, camphorous, resinous	
α-fenchene	HAD & FD powder	41,79,93,121,136	955	(A)	136.23	camphoreous	

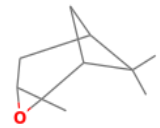
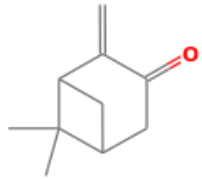
camphene	Oil from fresh leaves, rhizomes HAD & FD powder	41,67,79,93,121,136	957	(A)	136.23	woody, camphoreous	
α-myrcene	HAD & FD powder	27,41,68,79,93,121	981	(A)	136.23	spicy, woody, balsamic, resinous, terpenic, sharp	
β-pinene	Oil from fresh leaves, rhizomes, HAD & FD powder	27,41,93,136	985	(A)	136.23	fresh, piney, resinous, spicy	

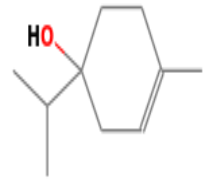
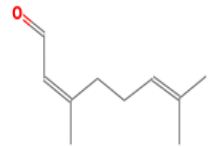
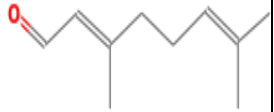
myrcene	Oil from fresh leaves, rhizomes, HAD & FD powder	27,41,69,93	993	(A)	136.23	spicy, woody, balsamic, resinous, terpenic, sharp	
δ-3-carene	Oil from fresh leaves, rhizomes, HAD & FD powder	77,93,105,121,136	1010	(A)	136.23	citrusy, piney, peppery, resinous, harsh	
α-terpinene	Oil from fresh leaves, rhizomes, HAD & FD powder	43,57,70,83	1022	(A)	136.23	woody, terpenic, spice, citrusy, camphoreous	

p-cymene	Oil from fresh leaves, rhizomes, HAD & FD powder	91,119,134	1030	(A)	134.22	woody, terpenic, rancid, spicy	
limonene	Oil from fresh leaves, rhizomes, HAD & FD powder	39,68,93,107,121,136	1035	(A)	136.23	tropical, citrusy, terpenic	
eucalyptol	Oil from fresh leaves, rhizomes, HAD & FD powder	43,81,108,139,154	1038	(A)	154.2	herbal, minty, camphoreus	

β-(E)-ocimene	Oil from fresh leaves, rhizomes, HAD & FD powder	41,53,79,93,121,136	1049	(A)	136.23	sweet, herbal, tropical, mango nuances	
γ-terpinene	Oil from fresh leaves, rhizomes, HAD & FD powder	4,77,93,121,136	1059	(A)	136.23	terpenic, herbal, spicy, tropical, lime, woody	
6,7-epoxymyrcene	Fresh and dried rhizomes	27,41,69,93,121,136	1093	(B2)	152.23	odourless	
perillene	Oil from fresh leaves, rhizomes, HAD & FD powder	41,69,81,136,150	1103	(A)	150	woody	

p-mentha-1,3,8-triene	HAD & FD powder	77,119,134	1123	(A)	134.21	terpenic, piney, woody, camphoreous	
allo-ocimene	Oil from fresh leaves, rhizomes, HAD & FD powder	79,105,121,136	1130	(A)	136.23	floral, citrusy, tropical, spicy	
neo-allo-ocimene	HAD & FD powder	79,105,121,136	1144	(B3)	136.23	floral, citrusy, tropical, spicy	

β-pinene oxide	HAD & FD powder	41,71,79,109,123,137	1157	(A)	152.23	herbal, piney, resinous, woody, camphorous	
pinocarvone	Oil from fresh leaves, rhizomes, HAD & FD powder	53,81,108,135,150	1168	(A)	150.22	balsamic, woody, earthy, minty	

terpinen-4-ol	Oil from fresh leaves, rhizomes, HAD & FD powder	43,55,71,93,111,136,153	1184	(A)	154	orange, floral, fermented, cheesy	
neral	Fresh and dried rhizomes	41,69,84,136	1244	(B4)	152.23	citrusy	
geranial	HAD & FD powder	41,69,84,136	1271	(B5)	152.23	citrusy	
perilla aldehyde	HAD & FD powder	39,54,68,79,108,122	1285	(B6)	150.21	woody, spicy, clove, citrusy	

perilla alcohol	Oil from fresh leaves, rhizomes, HAD & FD powder	41,68,79,93,121,136	1306	(A)	152.23	green, woody, spicy	
unknown volatiles	HAD & FD powder		799			-	
unknown volatiles	HAD & FD powder		902			-	
unknown volatiles	HAD & FD powder		1197			-	

a - Linear retention index on a HP-5MS column. b - (A), mass spectrum and LRI agree with those of authentic compounds; (B), mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited; [B1(Maccioni et al., 2007); B2-(Georgiou et al., 2010); B3-(Javidnia et al., 2006); B4-(Oliveira et al., 2006); B5-(Wang et al., 2005); B6-(Wang et al., 2006); B7-(Quijano et al., 2007)

Most of the compounds identified previously in the fresh rhizomes were seen in the dried samples as well, however, a significant reduction in volatiles occurred as a result of the drying process. These results highlight the heat sensitive nature of aromatic volatiles in mango ginger, which also aligns with similar studies conducted in a number of other aromatic plants. In sweet basil, the total quantity of volatiles were found to decrease considerably when subjected to convective drying at 40 °C, 50 °C and 60 °C and microwave drying at 240 W, 360 W and 480 W (Calín-Sánchez et al., 2012). A significant reduction in the concentration of monoterpenes when subjected to high temperatures of 30 °C and 60 °C were also identified in thyme and sage (Venskutonis, 1997). Comparative studies between drying techniques in parsley (Díaz-Maroto et al., 2002) reported that oven drying at 45 °C and freeze drying caused a significant decrease in the concentration of aroma compounds when compared to drying at ambient temperature.

3.3.2 Biochemical profile is more influenced by the combined effect of drying technique and storage duration

The results from this study showed that the combined effect of drying technique and storage duration significantly impacted a higher number of aromatic compounds when compared to those impacted by storage duration alone (Table 8). This impact also manifested differently across different chemical groups for each type of treatment. The change in relative abundance values of different chemical groups subjected to HAD and FD are discussed separately below.

Table 7 — Relative abundance of volatile compounds identified in the headspace of mango ginger using SPME GCMS from two different drying techniques (HAD - Hot Air Drying and FD - Freeze Drying) at three different storage times (M1 - Month 1, M2 - Month 2, M3 - Month 3)

		Mean relative abundance (AU) [i]								
		HAD [a]			FD [b]			P [j]		
	Compound	M1[c]	M2[d]	M3[e]	M1	M2	M3	Drying [f]	Time [g]	DXT [h]
Code	***Aldehydes***									
AL1	hexanal	2±0.58 ab	2±0.45 a	4±0.70 c	2±0.39 a	3±1.17 b	5±0.84 c	**	***	**
AL2	heptanal	0.34±0.20 b	0.08±0.17 a	0.60±0.07 c	0.25±0.10 ab	0.21±0.24 ab	0.41±0.06 bc	ns	***	**
AL3	benzaldehyde	0.16±0.12 b	0.05±0.09 a	0.01±0.04 a	nd	nd	nd	***	**	**
AL4	octanal	0.72±0.19 bc	0.57±0.14 ab	0.87±0.13 c	0.49±0.17 a	0.68±0.17 abc	0.49±0.06 a	***	ns	***
AL5	dodecanal	0.55±0.39 b	0.58±0.14 a	Nd	0.14±0.09 a	0.03±0.07 a	nd	**	***	**
	Total	**4.0**	**2.0**	**6.0**	**3.0**	**4.0**	**6.0**			
	Alkane									
ALA1	decane	6±1.32	6±0.99	5±0.70	6±2.44	6±0.89	5±0.73	ns	ns	ns
ALA2	undecane	4±1.19 d	3±0.63 ab	2±0.34 aa	4±0.87 cd	4±0.56 bc	2±0.37 a	ns	***	ns
	Total	**11**	**9.0**	**7.0**	**9.0**	**9.0**	**7.0**			
	Alkenes									
ALE1	dodecene	5±1.52 b	1±0.35 a	0.95±0.16 aa	4 ±1.14 b	0.62±0.21 a	2 ±3.89 ab	ns	***	ns
ALE2	tetradecene	2±0.54 b	0.52±0.18 a	0.36±0.11 aa	2±0.81 b	0.12±0.11 a	0.90±1.73 ab	ns	***	ns
	Total	**7.0**	**2.0**	**1.0**	**7.0**	**1.0**	**3.0**			
	Esters									
E1	bornyl acetate	0.19±0.11 ab	0.47±0.19 c	0.29±0.19 bc	0.09±0.08 a	0.10±0.10 a	0.17±0.11 ab	***	**	**
E2	methyl butonate	0.15±0.11	0.04±0.09	0.13±0.08	2±4.59	0.08±0.11	0.16±0.024	ns	ns	ns
E3	methyl dodecanoate	0.72±0.25 c	nd	0.04±0.13 aa	0.42±0.09 bc	nd	0.19±0.56 ab	ns	***	*
E4	isopropyl tetradecanoate	0.52±0.35 a	4±4.01 b	0.24±0.76 a	0.13±0.22 a	0.72±1.37 a	nd	**	***	*

	Total	**2.0**	**4.0**	**1.0**	**2.0**	**1.0**	**1.0**			
	Ketones									
K1	2-pentanone	0.21±0.07 ab	0.11±0.15 a	0.17±0.07 ab	0.23±0.11 ab	0.26±0.09 b	0.17±0.03 ab	*	ns	*
K2	2-nonanone	6±2.27 c	1±0.33 a	1 ±0.52 aa	5±1.09 c	3±1.41 b	2±0.64 ab	ns	***	**
K3	2-undecanone	1±0.37 b	0.09±0.10 a	0.14±0.092 aa	0.62±0.15 b	0.30±0.22 a	0.08±0.12 a	ns	***	**
	Total	**7.0**	**1.0**	**2.0**	**6.0**	**4.0**	**2.0**			
	Sesquiterpenes									
S1	E-caryophyllene	5±1.3 c	9±4.23 d	5±2.37 bc	2±0.54 ab	2±1.12 a	1±0.42 a	***	**	*
S2	α-humulene	0.99±0.24 b	2±1.13 c	0.87±0.501 ab	0.24±0.067 a	0.49±0.29 ab	0.25±0.08 a	***	***	**
S3	curzerenone	0.39±0.09 c	nd	0.03±0.05 aa	0.29±0.09 b	nd	0.04±0.06 a	ns	***	*
	Total	**7.0**	**11.0**	**6.0**	**3.0**	**2.0**	**2.0**			
	Terpenes									
T1	tricyclene	0.22±0.12 bc	0.27±0.14 c	nd	0.10±0.07 ab	0.22±0.13 bc	nd	*	***	ns
T2	α-thujene	0.22±0.15	0.21±0.15	0.12±0.07	0.13±0.04	0.13±0.08	0.16±0.07	ns	ns	ns
T3	α-pinene	5±2.08 bc	6±2.02 c	0.99±0.21 a	3±0.65 b	4±1.56 b	1±0.3 a	***	**	*
T4	α-fenchene	0.35±0.14 c	0.19±0.26 bc	nd	0.15±0.10 ab	0.19±0.12 bc	nd	ns	***	*
T5	camphene	2±0.81 c	3±0.84 d	0.72±0.09 a	1±0.23 abc	2±0.71 bc	1±0.26 ab	**	***	**
T6	α-myrcene	3±1 c	3±0.81 c	Nd	1.3±0.52 b	1.02±0.46 b	nd	***	***	***
T7	β-pinene	22±10 b	34±13 c	11±3 a	15±3 ab	15±7 ab	14±6 ab	**	***	**
T8	β-myrcene	455±198 cd	584±178 d	131±46 a	301±51 bc	212±86 ab	124±37 a	***	***	***
T9	δ-3-carene	0.96±0.38 b	1±0.28 b	0.25±0.12 a	0.49±0.10 a	0.39±0.13 a	0.23±0.10 a	***	***	***

T10	α-terpinene	0.88±0.37 b	0.96±0.27 b	0.39±0.11 a	0.53±0.12 a	0.47±0.18 a	0.576±0.168 a	**	**	***
T11	p-cymene	2±0.93 b	2±0.65 b	1±0.31 aa	1±0.16 a	1±0.44 a	1.03±0.25 a	***	***	**
T12	limonene	5±1.76 b	9±2.39 c	2±0.89 aa	3±0.54 a	3±1.23 ab	3±0.76 a	***	***	***
T13	eucalyptol	14±3 ab	37±9 c	13±3.27 ab	7±1.51 a	13±5 ab	18±5 b	***	***	***
T14	β-(E)-ocimene	13±4 b	21±7 c	2±0.78 a	9±1.53 b	3±1.59 a	0.99±0.36 a	***	***	***
T15	γ-terpinene	0.56±0.20 ab	0.64±0.12 b	nd	0.91±1.03 b	0.01±0.03 a	nd	ns	***	**
T16	6,7 epoxymyrcene	4±2 ab	6±2 b	3±1 a	3 ±0.82 a	3±2 a	5±1.5 ab	*	**	**
T17	perillene	14±5 b	13±4 ab	9±3 a	10±2 ab	12±4 ab	9±2 a	ns	**	**
T18	p-mentha-1,3,8-triene	0.50±0.20 c	0.53±0.11 c	0.01±0.03 aa	0.22±0.03 b	nd	nd	***	***	***
T19	allo ocimene	0.85±0.29 c	0.94±0.24 c	0.16±0.09 a	0.47±0.08 b	0.20±0.12 a	0.16±0.12 a	***	***	***
T20	neo-allo-ocimene	1±0.34 c	1.50±0.421 d	nd	0.733±0.135 b	0.093±0.166 a	nd	***	***	***
T21	β-pinene oxide	5±2 c	4±1.15 bc	nd	3±0.78 b	3±1 bc	0.88±1.36 a	ns	***	**
T22	pinocarvone	0.53±0.23 a	0.61±0.16 ab	0.40±0.10 a	0.38±0.11 a	0.51±0.23 a	0.88±0.3 b	ns	*	***
T23	borneol	0.40±0.13 ab	0.52±0.14 b	0.33±0.08 aa	0.42±0.14 ab	0.36±0.14 ab	0.38±0.15 ab	ns	ns	*
T24	terpinen-4-ol	0.47±0.13 b	0.71±0.19 c	0.34±0.12 ab	0.32±0.08 ab	0.30±0.13 ab	0.25±0.10 a	***	***	**
T25	neral	0.36±0.29 b	0.35±0.08 b	0.06±0.09 a	nd	0.04±0.08 a	0.02±0.05 a	***	**	**
T26	geranial	1.02±0.68 b	0.45±0.16 a	0.25±0.27 a	0.24±0.07 a	0.08±0.17 a	0.12±0.23 a	***	**	**
T27	perilla aldehyde	0.26±0.25 b	0.18±0.172 ab	0.17±0.1 ab	nd	nd	0.19±0.11 b	**	ns	**

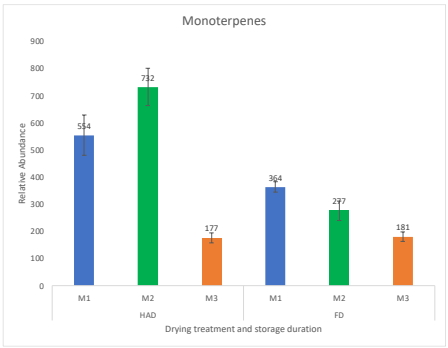
T28	perilla alcohol	1.9±1 c	1 ±0.44 bc	0.38±0.22 ab	0.25±0.04 a	0.44±0.28 ab	0.29±0.24 a	***	**	**
	Total	**554**	**732**	**177**	**364**	**277**	**181**			
	Unknown Volatiles									
UK 1	-	0.07±0.072 a	0.37±0.16 c	0.14±0.01 ab	0.16±0.04 ab	0.21±0.07 b	0.09±0.07 ab	ns	***	***
UK 2	-	6 ±5 b	5±2 ab	5±1 ab	3±0.34 a	3±2 a	4±1 ab	**	ns	ns
UK 3	-	0.61±0.24 c	0.26±0.46 ab	0.09±0.17 ab	0.31±0.07 bc	nd	0.15±0.17 ab	**	***	***
	Total	**7.0**	**5.0**	**5.0**	**3.0**	**3.0**	**4.0**			

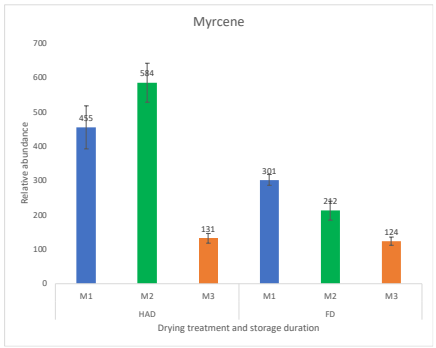
[a] HAD-Hot air drying (50 °C); [b] FD- freeze drying (-70 °C); [c] M1 (month 1of the storage time); [d] M2 (month 2 of the storage time); [e]M3 (month 3 of the storage time) [f] D Effect of drying; [g] D Effect of storage time [h] DXT Drying X Time interaction; [I] Estimated quantities (2mg) collected in the headspace of mango ginger samples (3mL) containing 1 mL of saturated calcium chloride filled up to 4mL, calculated by comparison with 100 µg/mL propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; relative abundance was calculated using the formula : (Peak area of the compound / Peak area of the internal standard) X Concentration of the internal standard; means of ten replicate samples are shown; nd, not detected; ns, not significant; [j] Probability obtained by ANOVA; * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

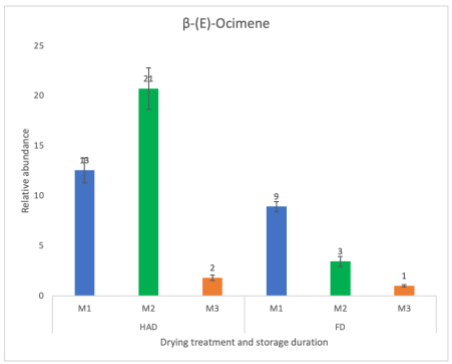
Terpenes and Esters

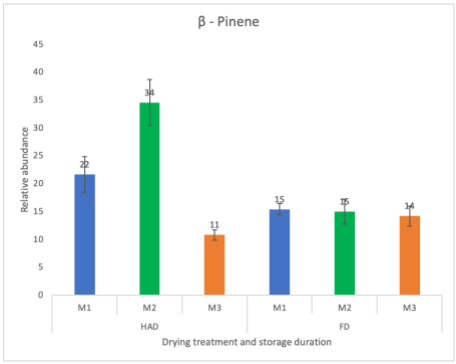
Terpenes (monoterpenes and sesquiterpenes) and esters showed a similar trend in terms of relative abundance values for each drying treatment across the three-month storage duration. Both of them showed a significant increase by M2, followed by a significant decrease by M3, when exposed to HAD. When exposed to FD, both groups of compounds significantly declined over M2 and M3 (Figure10). These changes were mainly driven by myrcene and E-caryophyllene, which were the compounds with the highest relative abundance values within monoterpenes and sesquiterpenes respectively.

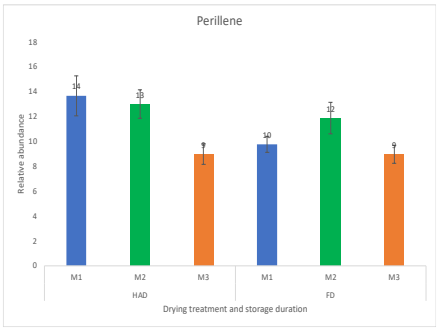
(A)

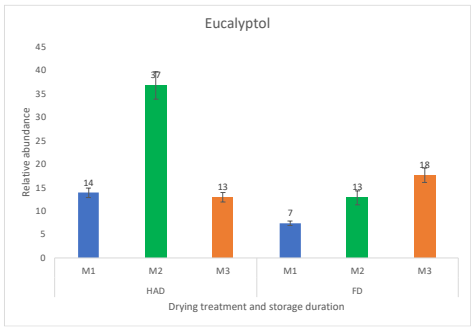

Monoterpenes
Relative Abundance
900
800
700
600
500
400
300
200
100
0
554
722
177
364
277
181
M1
M2
M3
M1
M2
M3
HAD
FD
Drying treatment and storage duration


Myrcene
Relative abundance
700
600
500
400
300
200
100
0
455
584
131
301
242
124
M1
M2
M3
M1
M2
M3
HAD
FD
Drying treatment and storage duration

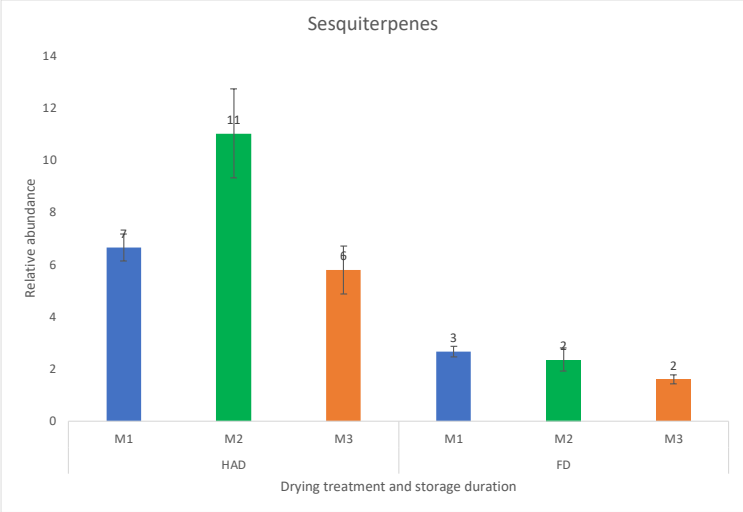

β-(E)-Ocimene
Relative abundance
25
20
15
10
5
0
13
21
2
9
3
1
M1
M2
M3
M1
M2
M3
HAD
FD
Drying treatment and storage duration


β - Pinene
Relative abundance
45
40
35
30
25
20
15
10
5
0
22
34
11
15
15
14
M1
M2
M3
M1
M2
M3
HAD
FD
Drying treatment and storage duration


Perillene
Relative abundance
18
16
14
12
10
8
6
4
2
0
14
13
9
10
12
9
M1
M2
M3
M1
M2
M3
HAD
FD
Drying treatment and storage duration


Eucalyptol
Relative abundance
45
40
35
30
25
20
15
10
5
0
14
37
13
7
13
18
M1
M2
M3
M1
M2
M3
HAD
FD
Drying treatment and storage duration

(B)



(C)

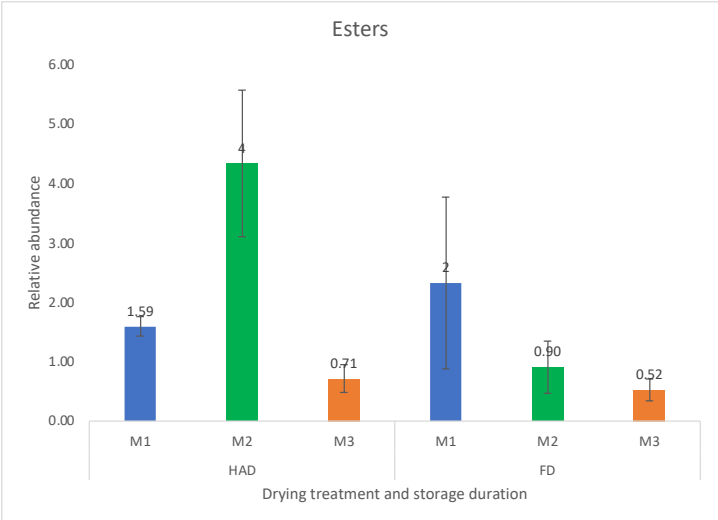


Figure 10. Changes in relative abundance of terpenes in mango ginger compared between HAD and FD across three different storage durations; (A [Changes in monoterpenes, including 5 major monoterpenes]; B [Changes in sesquiterpenes]; C [Changes in esters]). Error bars are calculated by using the standard error mean values; n = 10 HAD - Hot air treatment @ 50 °C, FD – Freeze drying treatment at – 55 °C to – 70 °C. Storage time(M1[month1], M2[month 2], M3[month 3]).

31 terpenes (28 monoterpenes and 3 sesquiterpenes) were identified in the biochemical profile. Monoterpenes showed an overall increase by M2 followed by a decrease by M3, when exposed to HAD. For FD samples, monoterpenes showed an overall decrease by M2 and a further decrease by M3. These trends are driven by the change in relative abundance patterns exhibited by most of the five major monoterpenes (myrcene, β-pinene, perillene, β-(E)-ocimene and eucalyptol) for

the respective drying treatment. Overall, there was a significant decrease in monoterpene concentration by M3 for both drying techniques (Figure 10A). Sesquiterpenes also showed a similar trend, mainly influenced the major component, (E)-caryophyllene (Figure 10B).

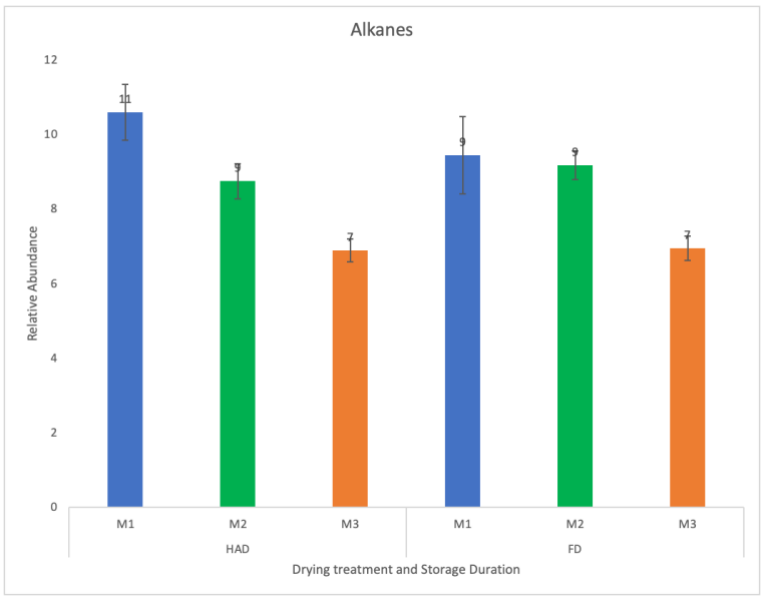
This finding aligns with drying studies conducted in other aromatic plants where a similar decrease in terpenes have been reported. Similar loss of terpenes like limonene, α-pinene and p-cymene when exposed to HAD (at 25 °C, 40 °C and 50 °C) and FD (at -25 °C) have previously been reported from GCMS studies in dill (Huopalahti et al., 1985). Studies conducted in spearmint have also reported that FD resulted in substantial losses of monoterpenes and sesquiterpenes, when compared to that of oven drying at 45 °C and air drying at ambient temperature (Díaz-Maroto et al., 2003). A similar decrease in monoterpenes have also been reported in Srilankan ginger when airdried in a hot air oven at 60 °C (MacLeod and Pieris, 1984).

Oxidation that occurs during storage has been attributed as one of the main reasons for the significant decrease in terpene concentration in aromatic plants, as reported in thyme (Venskutonis et al., 1996). Another reason has been attributed to losses occurring from evaporation due to the high partition co-efficient of compounds like α-pinene, myrcene and limonene in black carrots (Keskin et al., 2021) carrots (Rajkumar et al., 2017) mango (Bonneau et al., 2016) and red peppers (Jun et al., 2005) when subjected to various drying methods.

Esters also showed a similar trend to that of monoterpenes for HAD and FD treatments (Figure 10C). Alcohols were notably absent in the dried rhizomes, which correlated to the initial increase in the concentration of esters by M2 for HAD. Alcohols are the precursors to esters, so it may be hypothesised that all the alcohols present in the fresh samples were converted to esters during drying. This process of alcohol esterification has been demonstrated in peppers subjected to heat treatments (Ge et al., 2020) where the ester concentration increased significantly, promoting the fruity and green odour in roasted pepper seeds. In general, a significant decreasing trend by M3 was shown by esters for both drying techniques.

Alkanes and Ketones

(A)



(B)

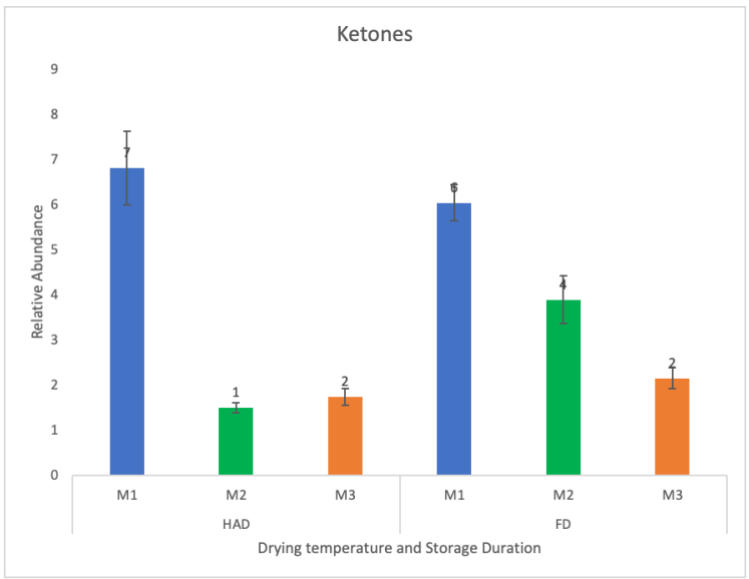


Figure 11. Changes in relative abundance of alkanes and ketones in mango ginger compared between HAD and FD across three different storage durations; (A[Changes in alkanes]; B [Changes in ketones]. Error bars are calculated by using the standard error mean values; n = 10. HAD - Hot air treatment @ 50 °C, FD – Freeze drying treatment at -70 °C. Storage time(M1[month 1], M2 –[month 2] M3 [month 3])

Alkanes and ketones showed similar changes in relative abundance across the storage duration for both drying treatments. Both of them decreased by M3 (Figure 11); however, ketones were

more significantly impacted by drying technique and storage duration, when compared to that of alkanes. Ketones showed a significant decrease by M2 for both HAD and FD samples. Of the three ketones identified, 2-nonanone and 2-undecanone significantly decreased by drying treatment and storage duration.

Of the two alkanes identified (decane and undecane), the significant decrease was only shown by undecane for storage duration (Table 7). It is worth noting that alkanes were newly formed compounds in the dried rhizomes. Formation of alkanes as a result of drying has been reported previously in Chinese black truffles as a result of oxidation of lipids (Ma et al., 2018) .This study also confirmed that non enzymatic browning (Maillard reaction) and Strecker reaction which occur as a result of heat treatments like drying and cooking act as catalysts for alkane production.

Aldehydes

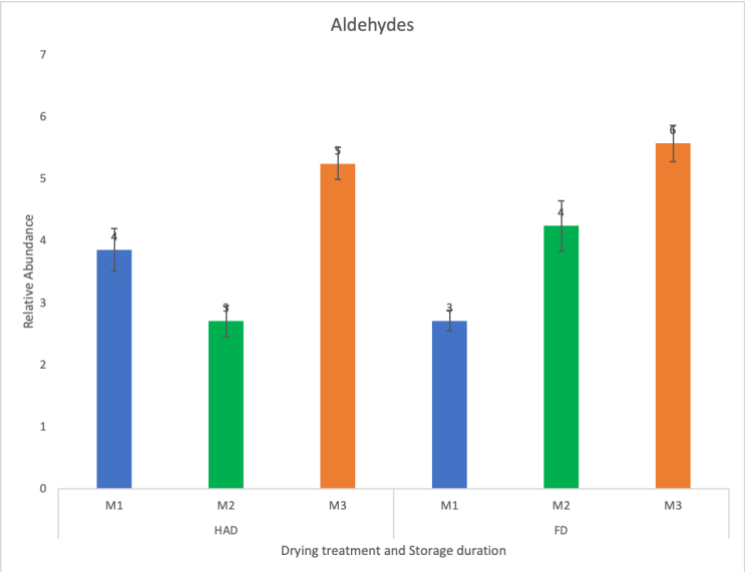


Figure 12. Changes in relative abundance of aldehydes in mango ginger compared between HAD and FD across three different storage durations. Error bars are calculated by using the standard error mean values; n = 10. HAD - Hot air treatment @ 50 °C, FD – Freeze drying treatment at -70 °C. Storage time(M1[month 1], M2 –[month 2] M3 [month 3])

Aldehydes showed a surprisingly opposite trend when compared to that of the other chemical groups. There was a significant increase in aldehyde concentration by the end of M3 for both drying techniques (Figure 12). Most of the six aldehydes identified were significantly impacted by drying treatment, storage duration and the combined effect of both factors (with the exception of heptanal and octanal). Although there was a significant dip in aldehyde concentration by M2 for HAD samples, they significantly increased in concentration by M3 and surpassed their M1 values.

Volatile aldehydes are mostly formed from fatty acid precursors through the action of enzymes such as lipoxygenase and hydroperoxyl lyase. The activity of these enzymes has been reported to increase at drying temperatures of 50 °C, and the high number of aldehydes created have been linked to off-flavours in boiled potatoes when stored (Petersen et al., 1999). The oxidation of alcohols also results in the production of aldehydes (Tojo and Fernandez, 2006), which would explain the complete absence of alcohols and the significant increase in aldehyde concentration in the dried rhizomes. In addition to hexanal and benzaldehyde, which are related to fruity notes in fresh rhizomes, and two new aldehydes octanal and dodecanal were identified in the dried samples. These new compounds are related to soapy and aldehydic aroma flavours (The Good Scents information system, http://www.thegoodscentscompany.com).

Alkenes

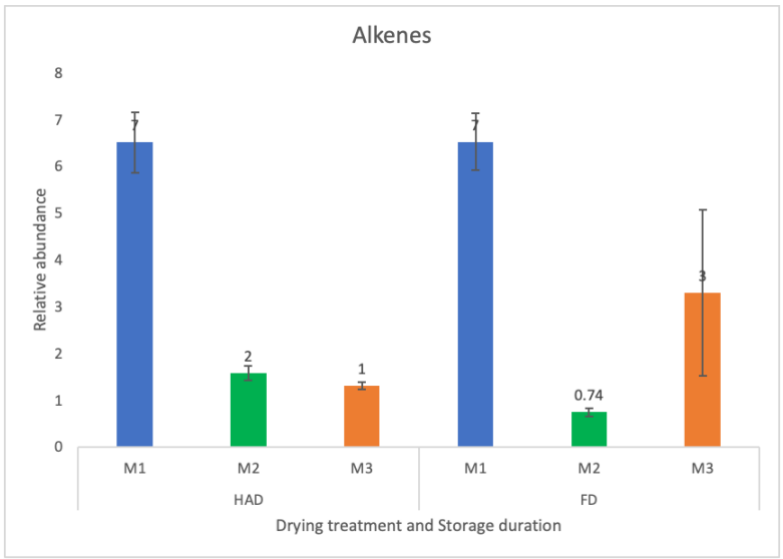


Figure 13. Changes in relative abundance of alkenes in mango ginger compared between HAD and FD across three different storage durations. Error bars are calculated by using the standard error mean values; n = 10. HAD - Hot air treatment @ 50 °C, FD – Freeze drying treatment at -70 °C. Storage time(M1[month 1], M2 –[month 2] M3 [month 3])

Drying technique had no significant impact on the two alkenes identified (dodecane and tetradecene). Both were significantly impacted by storage duration, in that a significant decrease in relative abundance was noted by M2 for both drying techniques (Figure 13). For samples subjected to FD, an overall increase was noted by M3, mostly due to the effect of dodecane which was able to skew the overall pattern seen in alkenes, owing to its high relative abundance value (Table 7).

General trend

The overall trend shows a significant reduction in the relative abundance values of total volatiles. This trend mirrored that of terpenes (Figure 14), as they accounted for 87 % to 93 % of total volatiles in HAD samples and 88 % to 92 % of total volatiles in FD samples.

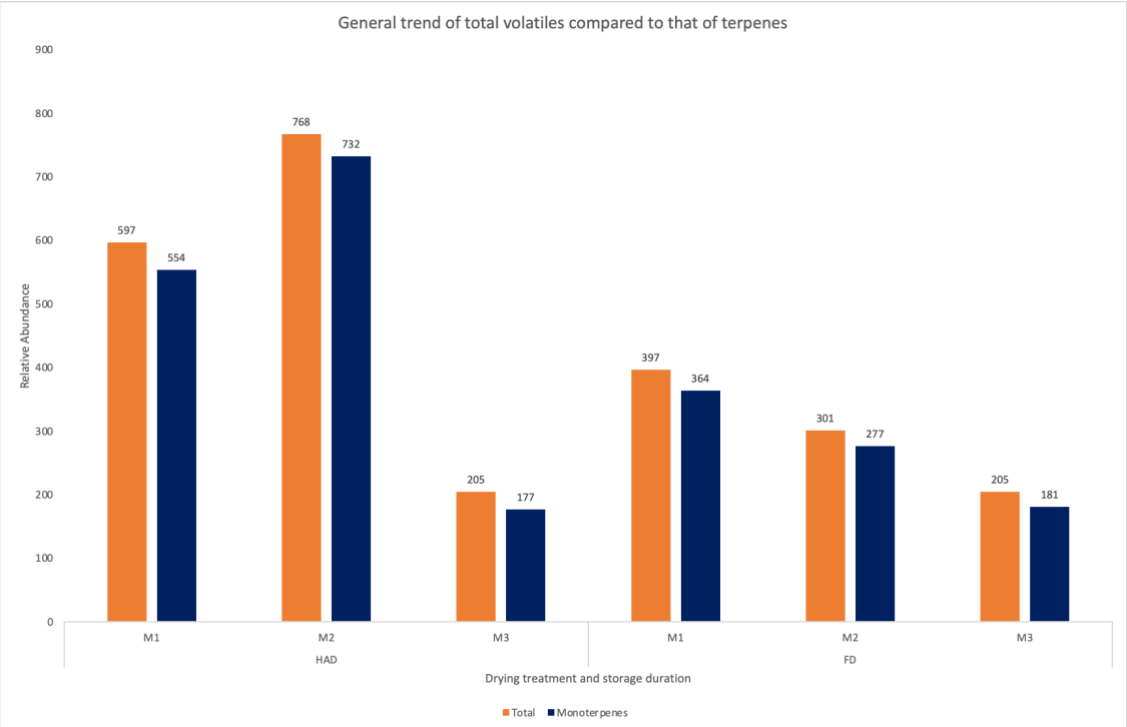


Figure 14. Changes in relative abundance of total volatiles compared to that of monoterpenes in mango ginger compared between HAD and FD across three different storage durations. Error bars are calculated by using the standard error mean values; n = 10. HAD - Hot air treatment @ 50 °C, FD – Freeze drying treatment at -70 °C. Storage time(M1[month 1], M2 – [month 2] M3 [month 3])

Several studies have shown that increased production of terpenes and C6 volatiles (alcohols, aldehydes, esters and ketone) is a defence response to protect against external biotic and abiotic stress factors (Ormeño and Fernandez, 2012; Mahizan et al., 2019). This would explain the formation of these compounds in HAD and FD samples in the current study as well, as the samples were exposed to similar stress factors when they were subjected to the various processing techniques (peeled, cut and exposed to ambient conditions for 2-3 hrs; exposed to a temperature of 50 °C for hot air drying; blast freezing at -18 °C ; prolonged freeze drying at -70 °C). The increased production of these compounds during the first two months can also be an adaptation process of tissue exposure to different temperature conditions and cell disruption during the different drying treatments.

Differences in the volatile profile due to different drying techniques and its effect on the volatile profile was explained in oven dried and FD guava powders (Taylor, 1998; Nunes et al., 2016). Their results found that terpenes were the predominant group of compounds in the dried produce. However, esters and aldehydes which give the characteristic flavour and aroma of fresh guava fruit, appeared to decrease after drying. The study explains that compounds with high vapour pressure and/or low liposolubility tend to be lost during drying, whereas compounds with low vapour pressure and/or high liposolubility tend to concentrate in guava powders. This would explain the relatively high percentage composition of terpenes in the current study as well.

There are reports that other reactions such as thermal degradation, unsaturated fatty acids degradation, Maillard reaction and enzymatic hydrolysis occur during the processing of spices (Luning et al., 1995), which can result in the production of diverse volatile compounds, especially terpenes. Such behaviour has been reported in fresh and dried peppers (Korkmaz et al., 2020; Korkmaz et al., 2017) as well as in herbs such as Lemon myrtle, Anise myrtle, and Tasmanian pepper leaf when dried, milled and stored recorded significant losses of volatiles (Chaliha et al., 2013) .Such reactions could also explain the formation of new compounds in the current study such as alkanes, alkenes and isomers like α-myrcene and neral, and the complete disappearance of alcohols in the dried product.

It can also be assumed that heat induced Maillard reactions or Strecker degradations as well as changes due to blast freezing followed by freeze drying could be responsible for the diversity of VOCs produced as well as various other enzymatic reactions taking place during the storage time of powdered samples. The occurrence of these reactions and their effect on altering the biochemical profile, aroma, colour and texture of food products have been explored in a number of studies (Bordas et al., 2004; Martins and Van Boekel, 2005; Jaeger et al., 2010; Rannou et al., 2016). The loss of volatile flavour compounds during dehydration have also been attributed to the inactivation of volatile-forming enzymes along with the loss of the precursors. Studies conducted in onions (Freeman and Whenham, 1975) on the activity of these enzymes have shown that a 90 % reduction of the alliinase enzyme occurred when subjected to HAD and a 45 % reduction was noted in FD samples.

Another reason for the variation of the volatiles could be due to wounding (by peeling and cutting) of samples prior to drying in the current study, exposure to different drying temperatures, oxidation followed by various enzymatic reactions, degradation and volatilization which continue during the storage time could also have had a cumulative effect on the volatile profile of the final produce. Wounding or injury damages membrane systems of cells (Bangerth, 1979; Mazliak, 1983) resulting in extensive enzymatic degradation of membrane components (Galliard and Matthew, 1976; Galliard et al., 1976; Galliard and Phillips, 1976; Wardale et al., 1978; Feys et al., 1980; Yapa et al., 1986), and the depletion of their lipid components. Loss of membrane lipid components is mediated by the action lipoxygenase activity which are involved in the creation of both desirable and undesirable aroma volatiles (Mazliak, 1983). These metabolic changes also include the accumulation of secondary metabolites leading to a decompartmentalization of enzymes and substrates, which occur to restore cell membranes that were damaged during wounding. The cumulative effects of all these enzymatic and non-enzymatic reactions and associated changes could have influenced the final volatile composition in HAD and FD powders.

3.3.3 Sensory Analysis

Aroma and flavour attributes of HAD and FD mango ginger samples were assessed by the sensory panel (Table 8). Of the 26 attributes identified from the powder and tea made from the dried samples, panellists identified significant differences due to drying treatments in eleven attributes such as powder colour, tea colour, texture, aroma attributes (piney, milky), and flavour attributes (fresh mango ginger taste, bitter, woody and after taste effects such as throat burning, throat drying, and salivating) in this trial.

Table 8 – Mean panel scores for 26 sensory attributes of mango ginger subjected to HAD and FD stored for three different durations (M1, M2 and M3).

Score [A]									
Code	**Attribute**	**Anchor point**	**HAD M1**	**HAD M2**	**HAD M3**	**FD M1**	**FD M2**	**FD M3**	**P [B]**
	Aroma of powder		**Score [A]**						
ap1	Fresh mango ginger	Mild to Extreme	44	46	46	42	48	37	ns
ap2	Gingery		20	26	24	19	20	15	ns
ap3	Turmeric		11	13	10	8.0	7.0	7.0	ns
ap4	Musty		5.0	8.0	3.0	3.0	9.0	8.0	ns
ap5	Piney		29 a	29 a	35 a	25 ab	27 ab	18b	**
ap6	Milky		0.8 b	3 b	0.0 b	5 a	4 b	18 a	**
ap7	Rubbery		0.7	0.0	0.8	1.0	2.0	0.4	ns
cp1	**Colour of powder**	Mustard yellow to pale yellow	27 b	22 b	29 b	67a	75a	80a	***
tp1	**Texture of powder [C]**	Grainy to floury	36 b	27b	36 b	73 a	81 a	83 a	***
ct1	**Tea colour [D]**	Light yellow to colourless	49 ab	37ab	30.4b	62 a	64 a	61a	**
	Aroma of tea								
at1	Boiled potatoes	Mild to Extreme	28a	42 a	27 b	31 a	27 a	22 a	ns
at2	Fresh mango ginger		12 b	16ab	26 a	11 b	15 ab	22 ab	*
at3	Citrusy		2.0	3.0	3.0	5.0	5.0	3.0	ns
at4	Gingery		13	13	15	8.0	8.0	9.0	ns
	Flavour of tea								
ft1	Fresh mango ginger	Mild to Extreme	19 b	24 ab	35 a	20 b	20 b	25 ab	ns
ft2	Sweet		12	13	12	11	14	9.0	ns

ft3	Bitter		7.0b	13 b	24a	9.0 b	8.0 b	14 ab	*
ft4	Citrusy		4.0	3.0	4.0	5.0	6.0	5.0	ns
ft5	Woody		10 ab	16 ab	20a	9.0 b	11ab	12 ab	*
ft6	Earthy		6.0	7.0	12	6.0	5.0	6.0	ns
ft7	Off flavour		0.0	2.0	4.0	0.0	0.1	5.0	ns
	Aftertaste effect								
ae1	Mouth drying	Mild to Extreme	29	27	30	28	23	27	ns
ae2	Mouth burning		11	14	16	7.0	6.0	8.0	ns
ae3	Throat drying		15ab	15 ab	24 a	13ab	9.0 b	11 b	*
ae4	Throat burning		14 b	11b	42a	8.0 b	6.0 b	7.0 b	***
ae5	Salivating		18b	23ab	33 a	19b	18. b	22ab	*

[A]: Means are from two replicate samples; differing small letters (a, b, c) represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$). [B]: Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5 % level; * significant at the 1 % level; ** significant at 0.1 % level; C, D – the intensity of these attributes for each sample was recorded by the assessors on a 0-100 point unstructured line scale; texture was rated from coarse to fine and colour was rated from dark yellow to light yellow. n=11.

The assessors were able to differentiate between HAD and FD samples, mainly due to differences in their aroma, flavour, texture and colour. Within the powder aroma attributes there was a marked difference for piney aroma, where HAD powders scored more compared to FD samples. For piney aroma, a maximum score of 34.7 was recorded in month 3 (the end of the shelf-life period). However, the panellists found no significant difference for fresh mango ginger, gingery, turmeric, and musty aroma between both the powders. The retention of these aroma characteristics, especially fresh mango ginger, gingery and piney can be attributed to the dominance of terpene compounds present in the rhizome as identified by instrumental analysis. The aroma attributes were maintained by both the powders until M3.

Milky and rubbery notes were the other two new aroma attributes associated with both the powders. A high score of 18.2 was identified for the milky note in FD samples by the end of the trial period, while this was absent in HAD powders by the end of the trial period. For both these attributes HAD samples scored lower than FD samples and showed no significant difference for rubbery notes.

Both treatments showed significant differences for powder colour, texture, and tea colour. The colour intensity of the powder ranged from mustard yellow to light yellow. HAD samples imparted a mustard yellow colour while FD samples imparted a light-yellow colour like that of fresh mango ginger. The significant difference in colour and texture can be attributed to differences in the drying techniques used; during HAD the samples turned darker and shrunk in size, leading to a harder texture at 50 °C, resulting in the dark colour and coarse texture. Whereas during FD the samples were kept fresh and cold under frozen conditions and sublimation could have resulted in the alteration of cell structure and pigmentation. All of these could explain the final lighter texture when compared to that of HAD samples. Similar results have also been reported in pumpkin, where HAD samples at 70 °C produced powders with darker colour while FD produced powders with a colour closely resembling that of fresh pumpkin (Que et al., 2008).

The tea prepared from both samples, after brewing 2 g of the powder for 5 minutes were assessed for colour. FD powder being light in colour was found to produce a colourless tea while HAD powder imparted a light-yellow colour to the tea when brewed.

Tea aroma attributes such as boiled potato, fresh mango ginger, citrusy and gingery were then scored. Both the powders when brewed gave a boiled potato aroma along with a mild fresh mango ginger. No significant differences were found between the powders for boiled potato aroma; however, this attribute had the highest score. This could be attributed to the relatively high starch content of mango ginger (45.64 % of dry weight) (Policegoudra and Aradhya, 2008) which could have imparted the boiled potato aroma when brewed in boiling water. Another reason could be the increase in aldehyde concentration across the storage duration, as confirmed in studies conducted in boiled potatoes (Petersen et al., 1999).

Only fresh mango ginger aroma showed significant difference between the treatments. A high score of 25.9 was recorded in HAD samples, indicating that such samples retained more aroma, and the higher score was recorded throughout the storage period. There were no significant differences in citrusy and gingery aroma for the tea from both the powders, however it was noted that the citrusy aroma was retained by FD powders while gingery aroma was retained by HAD powders by the end of the shelf-life period. Gingery aroma also had a higher score than citrusy aroma.

Tea flavour attributes such as bitter and woody notes recorded significant differences; both these attributes scored highly in HAD samples (24.4 and 20.0 respectively) and increased by the end of the shelf-life period. Other attributes such as fresh mango ginger, sweet, citrusy, earthy, and off flavours did not record a significant effect, but HAD powders scored highly for sweet and earthy flavour. Other flavour attributes like citrusy notes were perceived more in FD samples but didn’t show any significant effect across the trial period.

Both the powders scored highly for off-flavour by the end of the storage period. This could be attributed to the activity of spoilage micro-organisms which can work alongside enzymatic

changes resulting in an alteration of the aroma and flavour profile, as reported in storage studies using pine nut powder (Yang et al., 2021). Spoilage micro-organisms like firmicutes and cyanobacteria were responsible for inducing off flavour in pine nut powder as well an increase in hexanal concentration, which ultimately resulted in alteration of the flavour profile. The off-flavour produced by both powder samples could therefore be due to the spoilage micro-organisms as well as increased aldehyde volatiles.

As in the previous trial, throat burn, throat drying, mouth burn, mouth drying, and salivating were the after-taste effects assessed by the panellists. Of the five after taste attributes, throat burn (42.1) followed by salivating (32.5) and throat drying (23.7) were found to be significantly different. These aftertaste effects were prominent in HAD powders when compared to that of FD samples. It was also observed that the after-effect taste increased across the storage time in both the powder samples. The other two attributes such as mouth drying, and mouth burning did not show any significant differences between the two types of powders. But a high score of (26.5 and 8.1) for FD and (30.4 and 16.1) for HAD powers were recorded by the end of third month for mouth drying and mouth burning.

In stark contrast to the previous trial where mango ginger was provided in raw form to the panellists, it was noted in the current trial that the aftertaste effects did not mask the original fresh mango ginger aroma and flavour. The aftertaste attribute also scored much lower when compared to that of the previous trial. This could be due to the samples being presented to the panellists in the form of tea in which the harsh aftertaste compounds may have got altered. Another cause could be the loss of volatiles responsible for aftertaste effects during the drying process.

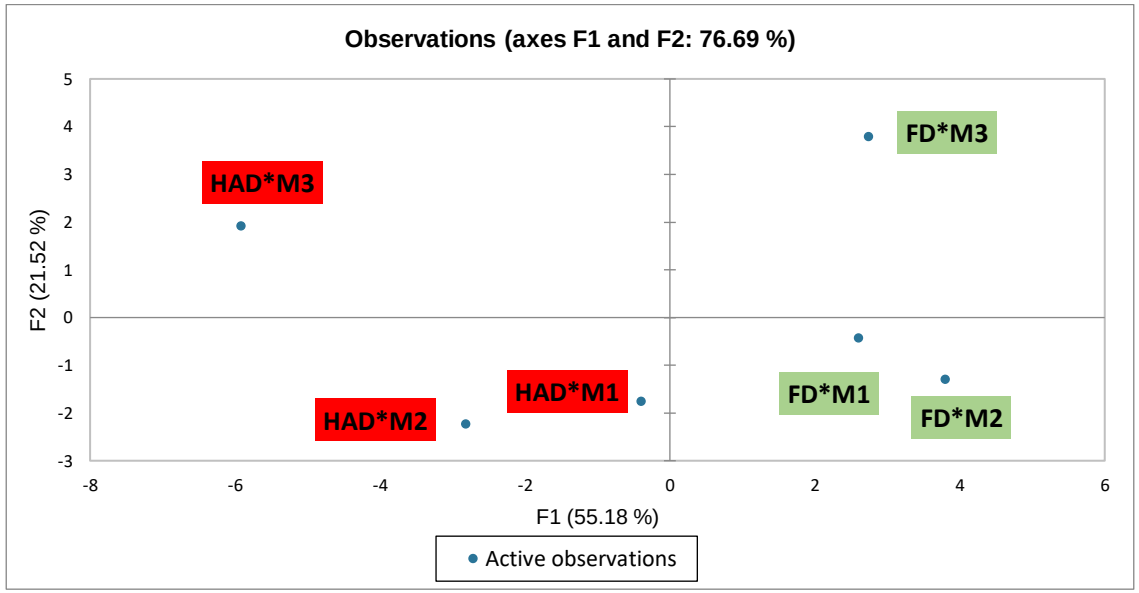
In general, the intensity of after-taste effects was found to be less in both HAD and FD powders. The fresh mango ginger aroma and flavour was retained across the storage period when compared to that of fresh rhizomes evaluated in the previous trial. It was also observed that the strong after taste effect was perceived in HAD samples towards the end of the trial period.

The impact of two different types of drying, its effects on the flavour volatile compounds and the associated changes in the sensory attributes were further evaluated by Principal Component Analysis (PCA) in which the various flavour compounds its correlation with sensory attributes and its association with the storage time were identified.

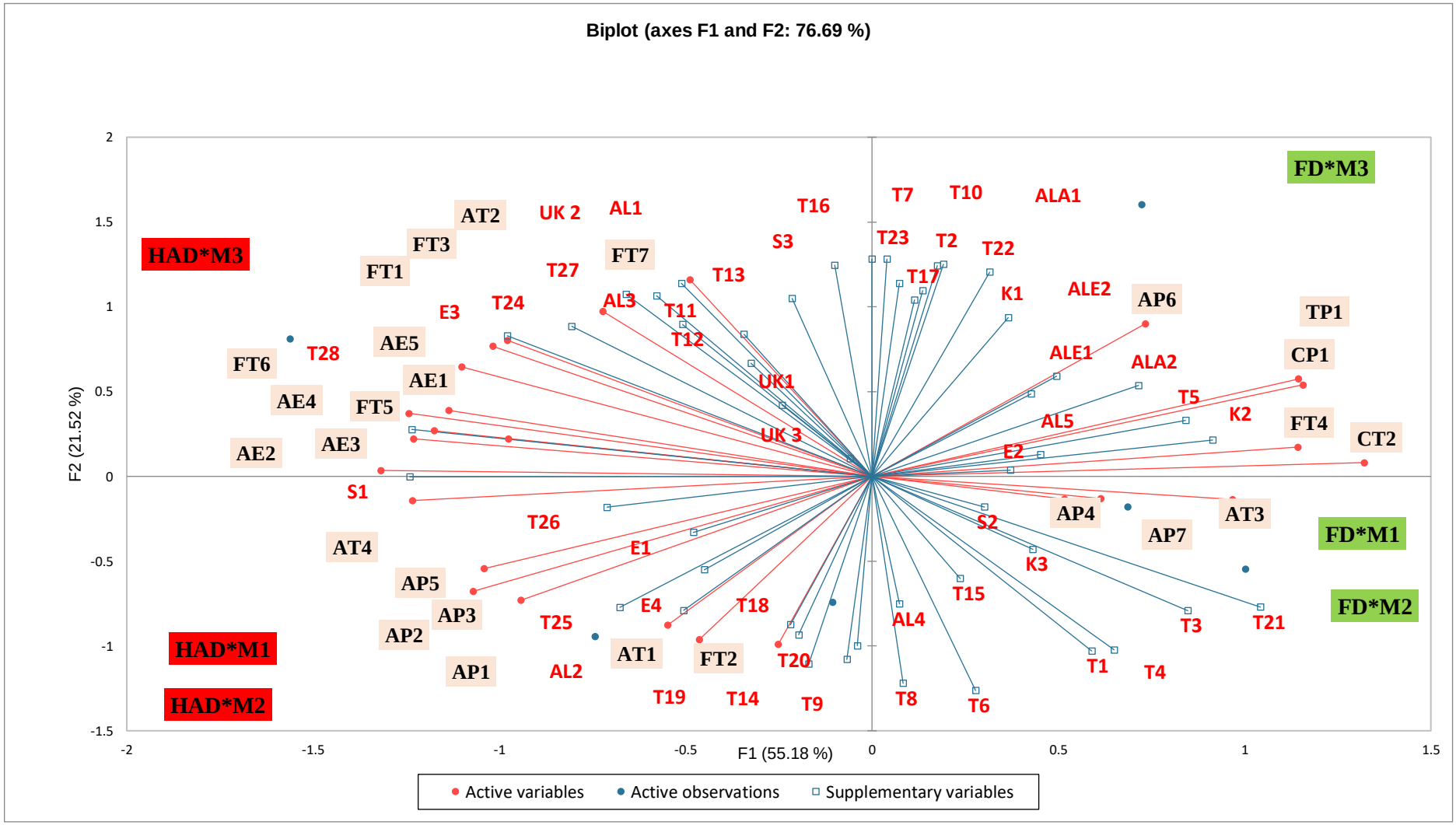
3.3.4 Principal Component Analysis

Principal component analysis (PCA) allowed the visual comparison of the volatile composition in the HAD and FD mango ginger powders to its organoleptic properties by overlapping the HS-SPME results and sensory data. The 50 VOCs identified were analysed with the aroma, colour, texture, flavour and aftertaste attributes to get an overview on how the variations in the sensory quality and chemical composition is affected by the different drying techniques and storage time. The PCA analysis gave an overview about the correlation of volatile compounds and its effect on various sensory attributes as shown in (Figure 15 A&B).

(A)


Observations (axes F1 and F2: 76.69 %)
F2 (21.52 %)
F1 (55.18 %)
HAD*M3
FD*M3
HAD*M1
HAD*M2
FD*M1
FD*M2
Active observations

(B)


Biplot (axes F1 and F2: 76.69 %)
F2 (21.52 %)
F1 (55.18 %)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
HAD*M3
HAD*M1
HAD*M2
FD*M3
FD*M1
FD*M2
AT2
UK 2
AL1
T16
T7
T10
ALA1
FT3
S3
T23
T2
T22
FT1
FT7
T27
T13
T17
K1
ALE2
AP6
E3
T24
AL3
T11
TP1
T12
AE5
ALE1
CP1
FT6
T28
ALA2
UK1
AE1
T5
AE4
FT5
K2
FT4
AL5
UK 3
CT2
AE3
E2
AE2
S1
AP4
AT3
T26
S2
AP7
AT4
E1
K3
AP5
T15
E4
T18
AP3
T25
AL4
T3
T21
AP2
AT1
FT2
T20
T1
T4
AP1
AL2
T19
T14
T9
T8
T6
Active variables
Active observations
Supplementary variables

Code	Volatile compound	Code	Volatile Compound
	Aldehydes	T14	β- (E)-ocimene
AL1	hexanal	T15	γ-terpinene
AL2	heptanal	T16	6,7 epoxymyrcene
AL3	benzaldehyde	T17	perillene
AL4	octanal	T18	p-mentha-1,3,8-triene
AL5	dodecanal	T19	allo ocimene
	Alkanes	T20	neo-allo-ocimene
ALA 1	decane	T21	β-pinene oxide
ALA 2	undecane	T22	pinocarvone
	Alkene	T23	borneol
ALE 1	dodecene	T24	teterpinen-4-ol
ALE 2	tetradecene	T25	neral
	Esters	T26	geranial
E 1	bornyl acetate	T27	perilla aldehyde
E 2	methyl butonate	T28	perilla alcohol
E3	methyl dodecanoate	UK 1	UK
E4	isopropyl tetradeconate	UK 2	UK
	ketones	UK 3	UK
K1	2-pentanone		**Sensory attribute- powder**
K2	2-nonanone	AP1	Fresh mango ginger
K3	2-undecanone	AP2	Gingery
	Sesquiterpenes	AP3	Turmeric
S1	E-caryophyllene	AP4	Musty
S2	α-humulene	AP5	Piney

S3	curzerenone	AP6	Milky
	Terpenes	AP7	Rubbery
T1	tricyclene		**Aroma and flavour - tea**
T2	α-thujene	AT1	Boiled potatoes (aroma)
T3	α-pinene	AT2	Fresh mango ginger (aroma)
T4	α-fenchene	AT4	Gingery (aroma)
T5	camphene	FT1	Fresh mango ginger (flavour)
T6	α-myrcene	FT5	Woody (flavour)
T7	β-pinene	FT6	Earthy (flavour)
T8	myrcene		**Aftertaste attribute - tea**
T9	δ-3-carene	AE1	Mouth drying
T10	α terpinene	AE2	Mouth burning
T11	p-cymene	AE3	Throat drying
T12	limonene	AE4	Throat burning
T13	eucalyptol	AE5	Salivating

Figure 15. Principal component analysis of HAD (Hot air treatment @ 50 °C), FD (Freeze drying treatment at -70 °C) samples of mango ginger kept at different time and showing correlation with volatile compounds and sensory attributes s (A) Projection of the samples; (B) Distribution of variables.(C) Compound codes appearing as in plot (B).

The results (Figure 15A) were visualised by means of biplot showing (F1) and (F2) which explained 76.69 % of variation, which is much higher than that recorded for fresh samples (57.37 %). This was because the panellists were unable to significantly distinguish the aroma and flavour components due to the masking caused by after taste effects in fresh samples. A large proportion of the variation between storage duration and volatiles were found in F1 (55.18 %), whereas F2 (21.52 %) mainly explained variation in the other flavour attributes associated with different drying methods.

A clear segregation between the different drying techniques with respect to aroma, flavour and aftertaste effects was noted from the biplot (Figure 15 B). There is a clear separation of HAD samples (HAD) and associated volatile flavour compounds towards the left side and the FD samples (FD) and the associated volatile flavour compounds towards the right side on the biplot. It was also observed that, there is a clear differentiation between the storage period, as the M1 and M2 (month 1 and month 2) for both the dried powders can be seen on the lower side of the quadrant and M3 (month 3) and the associated volatile compounds and sensory attributes can be seen on the top portion of the quadrant.

The prominent aroma attribute associated with FD powders was milky aroma (AP6) plotted on the top right-hand side of the biplot (Figure 15B); this was found to be positively correlated with the terpene volatile pinocarvone (T22) ($r=0.853$, $p<0.05$), and negatively correlated to fresh mango ginger (AP1) and piney aroma (AP5) ($r=-0.824$, $p=<0.05$; $r=-0.029$, $p=<0.01$) (Figure 15 C). This prominence of milky aroma also aligned with the sensory results which identified this attribute only in FD powders stored until M3. The correlation table with the r and p values are shown in Appendix 2.

Powder aroma attributes such as gingery (AP2) and piney (AP5) were found to have a strong positive correlation with each other ($r=0.836$, $p<0.05$; $r=0.831$, $p<0.05$) in HAD samples for M1 and M2. During the same storage duration, turmeric aroma (AP3) showed a strong correlation with boiled potato aroma (AT1) for tea prepared from HAD samples at M1 and M2 ($r=0.812$,

p<0.05). The terpene volatile neral (T25) also showed a strong correlation with turmeric aroma for these samples (r=0.830, p<0.05). These associations were confirmed by the sensory panel as they could only perceive ginger, piney, turmeric and boiled potato aroma along with the fresh mango ginger aroma (AP1) for these samples. Along with the boiled potato aroma of the tea (AT1), the volatiles such as β-(E)-ocimene (T14) (r=0.840, P=<0.05) and isopropyl tetradeconate (E4) (r=0. 880, p<0.05) was found to be strongly associated with HAD powders (represented on the left bottom quadrant of the biplot (Figure:15 B).

The fresh mango ginger aroma of tea (AT2) was perceived for HAD at M3 and was positively correlated (Figure 15 B&C) to terpene volatiles like p-cymene (T11) (r=0.832, p=<0.05), eucalyptol (T13) (r=0.858, p=<0.05), terpinene-4-ol (T24) (r=0.949, p=<0.01), and perilla aldehyde (T27) (r=0.873, p=<0.05). Aldehydes such as hexanal (AL1) (r=0.879, p=<0.05) and benzaldehyde AL3(r=0.841, p=<0.05) also showed a strong positive correlation. All of these sensory attributes were found to be aligned at the top left-hand quadrant of the biplot (Figure 10 B). The fresh mango ginger flavour (FT1) perceived for M3 samples was also found to be positively correlated with other attributes such as woody (FT5) (r=0.895, p=<0.05) and earthy (FT6) (r=0.922, p=<0.05) flavours, which agreed with the sensory panel scoring and comments.

As observed in chapter 2, a strong positive correlation of fresh mango ginger flavour (FT1) with after taste effects like throat burning (AE4) (r=0.858, p=<0.05) and salivating (AE5) (r=0.985, p=<0.01) was also noted in this trial. These sensory attributes were also found to be positively correlated with some terpenes, aldehydes and esters such as terpinen 4-ol (T24) (0.964, p=<0.01), perilla alcohol (T28) (r=0.863, p=<0.05), hexanal (AL1) (r=0.851, p=<0.05), benzaldehyde (AL3) (r=0.824, p=<0.05), and methyl dodeconate (E3) (r=0.988, p=<0.01). These flavour attributes and correlated volatiles were found to be arranged in the top left side of the biplot (Figure 15 B &C) which also align with the sensory results that these the after-effect tastes were perceived more during M3 storage period. These positive correlations seen in tea prepared form HAD samples agree with the sensory panel comments that they perceived earthy, woody and after taste effects along with the fresh mango ginger flavour for M3.

It was concluded from the PCA analysis that there were differences in the flavour volatile profile and associated organoleptic properties between HAD and FD samples. For HAD samples, the flavours like fresh mango ginger, piney, gingery, turmeric, and boiled potato were retained until M2. However, by the end of M3, the fresh mango ginger flavour was mostly associated with aftertaste effects such as throat burning (AE4), salivating (AE5), woody (FT5) and earthy (FT6). These associations were absent in FD samples; the only association of these samples were with milky aroma at M3. It can also be concluded that the current study results suggest both hot air dried and freeze drying are suitable for the production of mango ginger powders. Nevertheless, considering both economic viability and retention of aroma and flavour compounds, hot air drying and storage up to 2 months would be the best option for maintaining the shelf-life of these processed powders.

3.3.5 Other Physical properties

The powdered samples produced by hot air drying and freeze drying were analysed for their physical properties such as bulk density, solubility and absorption index, water activity, colour and final moisture content across the three-month storage period (Table 9).

Table 9 – Mean values for HAD and FD powder physical attributes at three different durations

Physical Attributes	HAD [a]			FD [b]			P [c]		
	M1	M2	M3	M1	M2	M3	D [d]	D [e]	D x D [f]
Bulk Density(w/v)	0.43 ±0.02b	0.47±0.02c	0.48±0.03c	0.20±0.01a	0.22±0.02a	0.22±0.01a	***	***	***
Colour Change (ΔE)	14±2.91b	8.0±1.75a	9.0±1.50	23±2.01d	16±1.07c	18±0.87c	***	***	ns
Moisture content (%)	8.0±0.37c	7.0±1.17c	7.0±0.68	4.0±0.64	4.0±0.47a	5.0±0.65b	***	***	***
Water Absorption(g)	7.0±0.34b	7.0±0.19b	7.0±0.28b	6.0±0.24a	6.0±0.56a	6.0±0.20a	***	ns	ns
Water Solubility (%)	7.0±0.84b	3.0±0.65a	4.0±0.42	8.5±0.83b	4.0±1.07a	4.0±0.814a	*	***	ns
Water Activity(a_w)	0.3±0.00d	0.23±0.01d	0.34±0.01e	0.02±0.00a	0.05±0.00b	0.15±0.02c	***	***	***

[a b] Mean values for HAD and FD samples-bulk density, colour change, moisture content, water absorption, water solubility and water activity moisture content and water activity in hot air- and FD mango ginger powders stored for a 3-month shelf-life period. Means labelled with letters are significantly different ($p < 0.05$) according to [c] Probability, obtained by ANOVA, that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level b; [d] Drying; [e] Duration; [f] DXD interaction between drying and duration interaction; means of ten replicate samples;

3.3.5.1 Bulk density (w/v)

Bulk density provides an indication of the packing and arrangement of the particles, as well as the compaction profile of a material (Mirhosseini and Amid, 2013). Bulk density indicates the porosity of a product, and it determines what type of packaging material to be used. The current study also recorded that the bulk density (Table 9), of HAD samples were significantly higher than that of FD samples. The bulk density values of the dried mango ginger powders ranged from 0.48 g/mL (for HAD) to 0.22 g/mL (for FD). Similar differences in bulk density values between HAD and FD have also been reported in pumpkin powders samples (Roongruangsri and

Bronlund, 2016), where the bulk density of powders produced at higher temperatures (0.9 g/mL) were higher than that produced at lower temperatures (0.1 g/mL). Powders with lower bulk density values can be packed in packaging materials that are less dense. FD involves crystallization of water in ice crystals which later undergo sublimation thus leaving the product porous and dried (Van Buggenhout et al., 2006). In HAD the samples undergoes dehydration and shrinkage which produces less porous samples (Ratti, 2001; Devahastin and Niamnuy, 2010).

3.3.5.2 Water solubility (%) and Absorption index (g)

One of the most important properties of a powder is its ability to dissolve in water. Most powdered products are expected to rehydrate quickly so that they can dissolve and disperse without forming any lumps (Hogekamp and Schubert, 2003). It was noted that HAD samples showed significantly more absorption (6.584 g) than FD samples (5.978 g). There was no significant effect due to storage time on water absorption. A different pattern for water solubility was observed. Solubility of powders was noted to be significantly impacted by storage duration. As the storage time increased the solubility of the powders was found to decrease. Water solubility index was high in both the powders (7.6 % for HAD and 8.4 % for FD) during the first storage month (Table 9).

Water solubility is an indicator of the extent of starch degradation, while the water absorption index indicates the extent of starch gelatinisation (Diosady et al., 1985; Hsu et al., 2003). Factors such as particle size, density, pH, composition, processing and storage conditions can also affect the water solubility of powdered products (Mirhosseini and Amid, 2013; Diosady et al., 1985). It has also been reported that hot air-dried pumpkin showed more solubility than FD because of more starch degradation during the heat treatment (Que et al., 2008). This would explain the high solubility of powders from both the HAD and FD treatments seen in the first month of storage in the current study.

3.3.5.3 Powder colour and Colour difference (ΔE)

The colour of food is an important quality parameter that can indicate changes in food quality arising from processing, storage, and other factors) (Aziah and Komathi, 2009).The total colour difference ΔE (Table 10), is a combination of parameters L*, A*, and B* values, and is a colorimetric parameter extensively used to characterise the variation of colours in foods during processing. In the present trial, it was noticed that both FD and HAD samples showed significant colour difference due to temperature. Due to high temperature, HAD samples were darker (mustard yellow) in colour than the FD samples. Similar changes in colour due to convection dried and in microwave-dried samples showing more redness were reported in garlic (Figiel, 2009). The colour of the powders was recorded across the storage time (Table 10).

Table 10 – Mean values for HAD and FD powder colour at three different storage durations

HAD [a]			FD [b]			P [c]		
L*	A*	B*	L*	A*	B*			
M1	M2	M3	M1	M2	M3	D [d]	T [e]	DXT [f]
70.91±2.93 b	-1.78±2.65 6c	21.23±4.84 d	81.16±2.01 c	-4.56±1.11 d	18.51±0.96 b	***	***	ns
66.67±0.156 ab	-1.29±0.2 ab	17.22±0.69 ab	77.24±0.39 ab	-4.05±0.32 ab	17.71±0.08 ab	***	***	ns
68.93±1.08 ab	-1.02±0.90 ab	19.08±1.60 ab	82.35±0.59 ab	-4.0±0.53 ab	18.29±0.18 ab	***	***	***

[a b] Mean values for the colour of HAD and FD samples stored for a 3-month shelf-life period. Means labelled with letters are significantly different ($p < 0.05$) according to [c] Probability, obtained by ANOVA, that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level b; [d] Drying; [e] Duration; [f] DXT interaction between drying and duration interaction; means of ten replicate samples; L* lightness of the powder, and ranges from black to white (0–100). A* negative value of a indicates green, while a positive number indicates red-purple colour. Positive B* indicates yellow and negative blue colour.

Both the drying temperature as well as storage duration has a significant effect on the colour of the powder. The L* value denoting the lightness of the sample was found to be significantly high in FD (L*= 82.35) and Hunter A* negative values representing redness (+) to green (-) was also found to be significantly high in FD samples (-4.003). Hunter B* representing yellow (+) to red

(-) was found to be significantly high in HAD samples (19.086) by M3. These findings aligned with the visual observations by the sensory panel in that FD samples were lighter than HAD samples.

FD samples showed the largest deviation in colour (ΔE -22.8) from that of the fresh material; this was noted in M1. HAD samples showed a lesser deviation during the same period (ΔE-13.54). However, from M2, the colour started to degrade for both samples until the end of the trial (M3). The sensory panel also noted that that the light-yellow colour of FD samples showed more similarity to that of fresh mango ginger samples.

During drying the HAD samples are directly exposed to oxygen under high temperature and this results in oxidation and degradation of compounds that could have led to changes in the colour pigments. FD samples are exposed to less oxygen and low temperature conditions; the resulting sublimation and shearing of the samples during freeze drying could have degraded the colouring pigments of the samples. Studies have shown (Weemaes et al., 1999; Que et al., 2008; Fellows, 2009; Workneh et al., 2014) that high drying temperatures, oxygen exposure, longer drying time can lead to degradation in carotenoids, degradation of chlorophyll and other pigments that can affect the colour and nutritive value of the final product.

3.3.5.4 Final moisture content (%) and Water activity (a_w)

Water activity and moisture content is one of the most critical factors in determining quality and safety of the food we consume. Other factors such as temperature can also influence the rate at which micro-organisms will grow in the product, but water activity is the most important factor controlling spoilage of foods. Most bacteria do not grow at water activities below 0.91, and most moulds cease to grow at water activities below 0.80. Water activity is the available water for microbial growth. In addition to influencing microbial spoilage, water activity can play a significant role in determining the activity of enzymes and vitamins in foods and can have a major impact on their colour, taste, and aroma. The moisture content also influences the texture, colour, and microbial shelf-life of foods. The safe moisture threshold boundary for grains, fruits and

vegetables, cash crops, and root and tuber crops is recommended to be within 6 % - 14 %, 0 % - 22 %, 0 % - 30 %, and 0 % - 10 % respectively (Etudaiye et al., 2009; Sanful and Darko, 2010; Afolabi, 2014).

In the current study (Table 9), the water activity was below 1 for both the samples. When compared between both the samples high water activity was recorded in HAD samples which increased as the storage time increased (0.286-0.341). FD samples showed the lowest activity (0.027) but was also found to be increasing (0.154) by the end of the storage period. In the study it was recorded that even though the water activity level in both the powders was within the safe levels the spoilage micro-organisms were detected in the samples.

In the current study, after preparing the batches of powdered samples they were sent to microbial analysis. They were tested for the presence of harmful pathogens *Clostridium, Salmonella, E. coli*, moulds and yeast powders. The lab results found that aerobic colony count was higher, but the Enterobacteriaceae count remained the same in HAD samples by M3 (Table 11); full report shown in Appendix 3). It can be assumed that these spoilage microorganisms and enzymatic changes would have resulted in producing more off flavour notes in HAD powders during the storage period, which was confirmed by the sensory panel. Both the aerobic count and Enterobacteriaceae count for FD samples were negligible by M3.

Table 11 – Microbial analysis values for HAD and FD powder at the beginning (M1) and end (M3) of the trial period.

Microbial analysis	M1		M3	
	HAD	FD	HAD	FD
Aerobic Colony Count 72h at 30 ºC	1.73x10^4 cfu/g	4.10x10^3 cfu/g	> 3.00x10^4 cfu/g	64 cfu/g
Enterobacteriaceae (presumptive)	> 1.50x10^3 cfu/g	320 cfu/g	> 1.50x10^3 cfu/g	10 cfu/g

The final moisture content in both the powders were less than 10 % but the moisture content was high in HAD (8.02 %) when compared to FD samples (3.79 %). Though HAD samples recorded high moisture content it was found to decrease as the duration of storage became more protracted. But an opposite trend was observed for FD samples, as the moisture content increased to (5.28

%) by the end of the storage time. This points to the inadequacies of the airtight plastic containers that were used to store the powdered samples and the need for further studies to evaluate other types of storage using vacuum packing to prolong the shelf-life of samples.

3.4 Conclusion

The current trial evaluated the differences in chemical composition and related sensory attributes of powdered mango ginger subjected to HAD (at 50 °C) and FD (-70 °C), with an intention to identify the ideal drying technique and storage period for future use as a value-added product and for commercial exploitation as aroma and flavour enhancers. A total of fifty volatiles were identified using SPME-GC-MS method. Both methods were found to conserve the major volatile components such as terpenes, and other minor groups such as sesquiterpenes, aldehydes, ketones and esters. From both the powder samples it was observed that alcohol groups disappeared completely and new compound groups such as alkanes and alkanes were found. Similar to fresh mango ginger samples, monoterpenes dominated the flavour compounds with myrcene, β-pinene and eucalyptol standing out as the major compounds.

The changes in the volatile profile of the powders and the consequent impact in the organoleptic properties were also assessed in this study by sensory analysis. Both the powders retained fresh mango ginger, gingery, turmeric, and piney aroma. But HAD samples were found to have more of these aroma attributes. Milky notes were found to be associated more with the FD powders. Though HAD samples scored more for the aroma and flavour attributes of tea they also scored more for undesirable attributes such as woody, earthy, and strong aftertaste effect- throat burning, throat drying followed by salivation towards the end of the storage period.

The association of various flavour components to the organoleptic properties were further analysed by PCA by overlapping the instrumental and sensory results. The PCA gave a clear separation between the flavour compounds, sensory attributes and the storage time effect associated with them. It was observed that HAD samples retained more of the flavour compounds for 2 months and the aftertaste effects were found to be increasing up until the third month of storage. The PCA also provides information regarding the different samples and the optimum storage period. From the sensory and PCA, it could be recommended that powders can be stored for up to 2 months to maintain desirable flavour and aroma qualities. Other physical properties

of the powder such as bulk density, water absorption, solubility, colour change, final moisture content and water activity across the 3-month storage period were found to be within acceptable limits.

The choice of drying technique used depends on a number of factors. From an economic feasibility perspective, HAD may be preferred over FD. However, the positive scores for physical properties such as bulk density in FD samples mean that they are more amenable to compact packaging, which could lower the overall costs of the supply chain cycle. These findings open up a number of opportunities for further studies to exploit commercially the benefits of essential oils found in mango ginger.

The focus of this study was limited to the volatile compounds in the biochemical profile; more work is needed for the non-volatile compounds as well to clearly understand the overall effect of processing on the flavour and aroma profile. The bioactive components such as total phenolics, antioxidants, starch content, and other nutraceutical activities should be tested before specific product development and commercialising them. The antimicrobial properties of the essential oils found in the fresh rhizomes should also be evaluated to determine whether they are retained in the dried powders.

There are other novel drying methods which combine two or more different drying methods that result in less energy requirement and shorter drying time, while maintaining most of the quality attributes. Methods such as solar assisted and microwave assisted hybrid drying techniques have been successfully used for herbs and spices (Jin et al., 2018). These have a number of advantages such as using renewable energy sources which are more economical (in the case of solar assisted drying) or to reduce drying times and accurately controlling the drying temperature (in the case of microwave assisted drying). Studies on different packaging materials to retain the fresh aroma and flavour also need to be conducted to identify user friendly and economical ways of distributing the product to the end customer.

CHAPTER 4

4. General discussion, limitations and future work

The final chapter of this thesis highlights the key findings and impact of this PhD project along with suggesting future avenues for research. The first part of the research focused on understanding the volatile profile of fresh mango ginger and the effect of different storage temperatures on the volatile profile of mango ginger when it was stored for a one-month period. The second part was aimed at determining the effect of different drying methods on the content and composition of volatile profile over a three-month shelf-life period. Sensory profiling was also done in both fresh and dried samples and across both storage durations to evaluate the changes in the VOC profile and associated changes in the organoleptic properties.

4.1 Why this study was initiated

Mango ginger is a relatively unknown member of the Zingiberaceae family and is cultivated in smallholdings in a few countries in southeast Asia. The rhizome has morphological similarities to ginger and releases the aroma and flavour of green mango when crushed. Previous studies conducted on mango ginger have focussed primarily on identifying the essential oil components that make up its biochemical profile. Literature on the effect of post-harvest practices and their impact on the aroma and flavour properties of mango ginger is sparse. As a result, there is a noticeable knowledge gap among key players (farmers, transporters, wholesalers and retailers) around providing optimal post-harvest conditions to prolong the shelf-life of harvested rhizomes.

The first phase of this study was aimed at improving the current practice prevalent among wholesalers and retailers of storing mango ginger rhizomes for a short period without undergoing any temperature regulation. The intention of the second phase was to determine whether commercial post-harvest treatments, such as drying, followed by long term storage were feasible options for mango ginger, with a view to exploring new avenues for this crop through commercial use of the dried powder. This is the first report that analyses the combined effects of drying

techniques and storage duration on the organoleptic properties of mango ginger rhizomes. To this purpose, this study explored the effect of three storage temperatures (8 °C, 15 °C and 22 °C) across three storage durations (1, 15 and 29 days) during its first phase. The analysis was extended to its second phase by utilising two drying techniques (hot air drying and freeze drying) and storing the powders for three different storage durations (1, 2 and 3 months).

4.2 Study findings

4.2.1 Terpenes account for a significant proportion of the volatile compounds in mango ginger

GCMS analysis used in this study confirmed that the biochemical profile of mango ginger is made up of a variety of chemical groups such as terpenes, alcohols, aldehydes, ketones and esters. Monoterpenes accounted for a significant proportion of the essential oil in the fresh rhizome and are responsible for the unique aroma of mango ginger, as noted in a number of previous studies (Gholap and Bandyopadhyay, 1984; Rao et al., 1989; Padalia et al., 2013). Although the relative abundance of other chemical groups was significantly lower than that of monoterpenes, they play an active role in imparting the fresh, floral and spicy notes of the essential oils.

4.2.2 The effect of storage temperature and duration on the volatile profile of mango ginger

SPME GCMS results presented in the first phase of the study conclude that changes in the biochemical profile of mango ginger are significantly impacted by differences in storage duration, mostly due to its effect on monoterpenes. A significant increase in the relative abundance of monoterpenes was noted by day 15, followed by a significant decrease by day 29. Myrcene, β-(E)-ocimene, β-pinene and eucalyptol retained their higher relative abundance values when compared to other compounds, across all three treatments. The highest relative abundance was noted for samples stored at 15 °C for two weeks. Results from the sensory analysis also confirmed the overpowering taste of terpenes for all treatments. Principal Component Analysis results confirmed a strong positive correlation between the flavour and aroma characteristics of green mango and mild turmeric for samples stored at 15 °C for two weeks. By the end of the shelf-life trial period (day 29), a clustering of after taste effects like throat burning, mouth drying, and mouth burning was identified for all temperature treatments.

It may be hypothesised that the initial increase in terpenes by Day 15 is a key indicator of senescence. The role of terpenes like β-pinene, β-terpinene, fenchyl acetate, camphene and δ-3-

carene in post-harvest senescence has been proven in previous studies (Chaimovitsh et al., 2010). The subsequent decrease in terpene concentration can be attributed to cell membrane destruction as part of the senescence process resulting in the loss of VOCs. The above results lead to the conclusion that storage at 15 °C for two weeks is the optimal combination of temperature and duration to retain the aroma and flavour characteristics of fresh mango ginger.

4.2.3. The effect of drying technique and duration on the volatile profile of mango ginger

The combined impact of drying technique and storage duration was found to have a significant impact on a greater number of aromatic compounds, when compared to the impacted by either of the factors separately. Chapter 3 shows that there were significant changes in trends identified within each chemical group as well. Terpenes, the dominant group of volatile compounds, significantly increased by the second month, which was followed by a significant decrease by the third month for hot air-dried samples. However, for those samples subjected to freeze drying, a steady decline was noted in the relative abundance of terpenes by the end of the third month. New compounds like alkanes and alkenes were identified and alcohols were completely absent in the dry samples.

The different trends identified between chemical groups can be attributed to the accumulation of secondary metabolites as a result of extreme wounding from peeling and slicing of the rhizomes. This leads to a decompartmentalization of enzymes and substrates to assist in repairing the damaged cell membranes. Once the samples are exposed to drying, heat induced Maillard reactions and Strecker degradations occur and continue throughout the storage period. The cumulative effects of all these reactions results in the loss of volatile forming enzymes and the required precursors resulting in the overall decrease of volatiles by the end of the storage period. These assumptions concur with previous studies mentioned in Chapter 3 which explain the creation of diverse volatile compounds during drying.

The sensory panel was able to identify fresh mango ginger aroma in hot air-dried samples, along with gingery and piney notes up to two months of storage. However, in freeze dried samples,

only a milky aroma was identified throughout the storage period. Hot air-dried samples were characterised by after taste effects like throat burning and salivating towards the end of the storage period.

Principal Component Analysis also confirmed a clear distinction between hot air- and freeze-dried samples. A strong correlation of green mango, mild turmeric aroma and flavour compounds was observed for hot air-dried samples by the second month. However, by the third month, a significant clustering of most of the aftertaste effects like throat burning, and salivating was seen. Strong correlations between the aroma and flavour attributes were absent in freeze dried samples throughout the storage duration. The above results lead to the conclusion that hot air dried samples stored for two months is the optimal combination of drying and duration to retain the aroma and flavour characteristics of dried mango ginger.

4.2.4. Practical implications of this study

The ideal storage temperature for harvested mango ginger rhizomes is 15 °C, as confirmed by this study. However, maintaining this temperature throughout the supply chain cycle is one of the main challenges that post harvesters will have to overcome, as the current practice involves bulk transportation of rhizomes across varying temperatures ranging from 7 °C to 28 °C (Figure 1). This process is followed purely for economic reasons as mango ginger currently does not have a market presence that would justify the costs involved in creating a tailor-made supply chain cycle with optimal temperature conditions.

Fresh mango ginger rhizomes may be subjected to a pre-harvest and post-harvest treatment of calcium chloride ($CaCl_2$) to improve the quality of shelf-life. This approach has proven successful in fruit crops and helped in delaying ripening, reducing respiration and in slowing down senescence processes. This treatment has also been able to reduce fungal infections in ginger (Bhai et al., 2019) and delay softening of strawberry fruits by maintaining the structural integrity of cell walls (Lara et al., 2004). $CaCl_2$ is also a cost-effective salt which is soluble in water

(Senevirathna and Daundasekera, 2010) and can therefore be easily used throughout the supply chain, irrespective of location.

Dried mango ginger rhizomes have yet to make their mark in the pharmaceutical and food industries in the UK, for the same economic hurdles faced by the fresh rhizomes. Despite being an excellent source of myrcene, the cost of creating a commercial drying process in the UK would far outweigh the returns. As this study has confirmed that HAD is better at retaining the VOCs in the rhizome. A better approach, however, would be to complete the drying process at the point of harvest itself, as this would normally be in countries like India, Pakistan and Malaysia where costs can be kept low. The resulting moisture loss from drying also reduces transportation costs during its journey from the point of harvest to the final consumer market in the UK. Alternative drying techniques like solar assisted drying have proven to be commercially feasible methods in a number of spices and herbs like turmeric and coriander (Parveen et al., 2014) . Similar approaches in reducing the barriers to entry of a relatively unknown spice like mango ginger should be considered to enable its transition from a domestically used culinary ingredient to a commercially used raw material for the food and pharmaceutical industries.

4.3 Limitations of the study

Certain limitations were encountered in this study, as discussed below.

4.3.1 The novelty of exotic flavours and the relative lack of experience in the sensory panel

The flavour and aroma attributes exhibited by fresh mango ginger was a novel experience for most members of the sensory panel. Owing to the COVID-19 pandemic, transportation delays resulted in the non-availability of natural reference materials like mature green mangoes and artificial ones like myrcene, β-(E)-ocimene and δ-car-3-ene to assist the detection of the unique flavour of mango ginger. All of this made it difficult for the panellists to familiarise themselves with some of the characteristic flavours and aroma attributes of mango ginger. Consequently, the panellists not being able to identify significant differences in flavour and aroma arising from different temperature treatments. Instrumental analysis has therefore been relied upon as a more reliant measure of identifying significant changes in the biochemical profile for this study.

4.3.2 The method of presentation of samples for sensory scoring

In the first phase of this study, raw mango ginger rhizomes were provided to the panel for scoring the sensory attributes. As in the case of most spices, the after-taste effects of raw samples are perceived very strongly, when compared to the inherent flavours. As a result, these after taste effects were able to mask the underlying flavours, especially in samples that had been stored for more than two weeks. This was mostly due to the high relative abundance values of terpenes when compared to other compounds that constituted the biochemical profile.

4.3.3 Biological replication and control samples

While conducting the second phase of this work (Chapter 3), the method used for sample preparation only allowed for technical replications and excluded biological replications. Additionally, control samples were also not analysed at Month 0 (i.e., immediately after the preparation of the powdered samples).

4.4 Recommendations for future work

The following recommendations are made based on the results of this study:

1. Several volatile compounds were identified in the headspace of mango ginger, all of which, in combination, contribute to the complex flavours and aroma of the rhizome. However, the perception of most of these odour compounds by human senses is unknown and their contributions to flavour are not fully understood. Although volatile compounds in the headspace can be identified using headspace-solid phase microextraction (HS-SPME) analysis, gas chromatography-olfactory (GC-O) analysis will need to be conducted in order to determine how the aroma of individual chemical groups are perceived by humans. In GC-O analysis, trained assessors are used for detection and verbal description of volatile aromatic components by scoring the odours on a scale (e.g., if using a seven-point-scale line, '3' would denote weak odour, '5' would be used for medium odour and '7' for strong odour). n-Alkanes C7 – C30 would then be analysed to obtain Linear Retention Index values for the volatile components (Lignou et al., 2014).
2. To identify and estimate the most dominant and odour-active compounds in mango ginger aroma extract dilution analysis (AEDA) can be carried out. This involves repeat analysis of several serial dilutions of the mango ginger extract by GC-O until only the most potent aroma compounds are detected in the extract; these compounds are then regarded as the most important contributors to mango ginger aroma. These techniques can be complemented with sensory panel assessments alongside an original mango ginger sample to determine if all compounds contributing to mango ginger flavour have been identified. Any change in the flavour compounds during storage and drying can be a useful indicator of changes in their volatile profile which will help to find the ideal flavour and aroma shelf-life of mango ginger.
3. This study has focused only on the volatile components of the biochemical profile of mango ginger. In order to get a comprehensive insight into the aroma and flavour profile of mango ginger, the contribution of non-volatile compounds should also be considered.

Studies in ethanol extractions of mango ginger (Siddaraju and Dharmesh, 2007) have identified phenolics such as caffeic acid, gentisic acid, ferulic acid, coumaric acid, gallic acid and cinnamic acid, but their aroma and flavour signatures have not been reported. Extraction using different solvents and Liquid Chromatography Mass spectrometry (LC/MS) analysis to determine the relative abundance of these non-volatile components along with the use of High-Performance Liquid Chromatography to determine the role of sugars such as fructose, glucose and sucrose can also be combined with sensory analysis to get a better understanding of the contribution of the compounds to the organoleptic profile.

4. Owing to the challenges faced by the sensory panel in identifying the flavours masked by after taste effects, consideration should be given to presenting samples in other than a raw form. For example, presenting the samples on different food matrices may help to balance out the aroma, flavour and after-taste effects for better detection and evaluation by sensory panellists. These evaluations can then be used to conduct consumer trials to determine the acceptance of the flavour and aroma attributes of mango ginger by the general public.
5. The drying techniques employed by this study ultimately resulted in a reduction in the overall VOC concentration. Novel and efficient drying methods which combine two or more different drying methods that result in less energy requirement and shorter drying time, while maintaining most of the VOC composition need to be investigated. Techniques like vacuum drying need to be explored by several initial trials to optimise conditions such as temperature, drying time and pressure to achieve and end product with desirable organoleptic properties and shelf-life.
6. The second phase of this work did not include biological replications or the analysis of a control sample. In order to evaluate the variability within the samples, both biological and technical replications should be employed. Whereas in this study, samples were selected separately from batches assigned for M1, M2 and M3, the approach should be changed to obtain samples from all batches equally and subjected to both instrumental

and sensory analysis. A control sample at Month 0 (immediately after the preparation of the powdered samples) should also be used to analyse the variability of the control sample between M1, M2 and M3.

4.5 Contribution to the industry

1. Chapters 2 and 3 confirmed that monoterpenes were the dominating constituents of the biochemical profile of mango ginger and that a high relative abundance was noticed for myrcene, in both fresh and dried produce. This identifies mango ginger as a natural source for extracting myrcene, which has been proven to have anticancerous and anti-aging properties (Bai and Tang, 2020; Surendran et al., 2021). Therefore, further research needs to focus on identifying cost-effective and sustainable methods of extracting myrcene from mango ginger for commercial use in the pharmaceutical and cosmetic industries.
2. Bioactive compounds rich mango ginger powders can be used as a functional ingredient as a substitute in various foods, such as ice cream and baking products. Their use as shelf-stable ingredients for food products like health drinks and marinades need to be explored to determine whether they can be used to meet the ever-growing demand for natural flavour additives. Similar use of fruits and vegetable powders as functional and food additives have been reported (Nindo et al., 2003; Camire et al., 2007). Commercial exploitation of these properties in the food industry needs to be explored in further studies.
3. Essential oils have been used widely in integrated pest management programmes with the aim of reducing the use of toxic chemicals (Zhang *et al.*, 2011b). Compounds such as α-pinene and dodecanol, were found to improve the effectiveness of pheromone traps used to control and disrupt the mating process of pests, ultimately reducing pest infestation (Kong et al., 2009; Wang and Chi, 2013). Studies have also proven the insecticidal and anti-oviposition properties of mango ginger against the pulse beetle (*Callosobruchus chinensis* L.) infesting green gram (*Vigna radiata)* (Ahmed and Ahmed, 1992). Mango ginger, therefore, has great potential in forestry and agriculture to produce pesticides that are environmentally safe.

4.6 Conclusion

The results obtained from this study have advanced our understanding of the relationship between different storage temperatures and the effect of different drying on the volatile composition and the resulting sensory profile of mango ginger. The study supports the finding that identifying an ideal storage temperature and duration is elementary to maintain the flavour life of mango ginger. The study also found that drying reduced the volatile components in mango ginger, but the characteristic aroma and flavour was retained for up to two months with hot air drying. This study opens further avenues for growers/processors of mango ginger to exploit its distinct green mango, mild turmeric aroma and flavour for use as natural flavouring agents in the food industry. Commercial extraction of myrcene from fresh and dried rhizomes also promises a natural source that can be used in both the pharmaceutical and cosmetic industries.

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Appendix 1 – Correlation matrix (Pearson) between sensory attributes and instrumental analysis in fresh mango ginger

Variables	A-GF	A-F	A-GM	A-MTU	A-SO	A-PI	F/T-SW	F/T-BT	F/T-GF	F/T-MTU	F/T-PI	F/T-SO	AE-TB	AE-SAL	AE-MD	AE-SO	AE-BI	AE-MB
A1	0.426	0.021	0.458	-0.023	-0.552	0.620	0.025	-0.087	**0.809**	**0.753**	**0.787**	**-0.677**	-0.603	**-0.760**	-0.255	-0.276	0.374	**-0.714**
A2	-0.404	-0.344	-0.355	-0.377	-0.240	-0.015	0.065	0.267	-0.155	-0.198	-0.080	-0.221	0.208	0.433	**0.732**	-0.515	0.190	-0.159
A3	-0.003	-0.048	-0.009	-0.438	-0.522	0.373	-0.110	0.181	0.415	0.225	0.482	**-0.784**	-0.278	-0.209	0.397	**-0.681**	0.291	-0.563
A4	-0.518	-0.363	-0.470	-0.567	0.103	0.038	0.526	-0.360	-0.213	-0.161	0.004	-0.457	-0.207	-0.058	0.427	-0.490	-0.452	-0.251
A5	0.500	0.145	0.521	0.097	-0.312	0.568	0.046	-0.317	**0.715**	**0.668**	**0.688**	-0.593	-0.624	**-0.851**	-0.559	-0.090	0.088	-0.616
A6	-0.178	-0.195	-0.238	0.172	0.473	0.130	0.601	-0.403	-0.237	-0.148	0.007	0.397	0.148	-0.118	-0.421	0.482	-0.509	0.199
AL1	-0.639	-0.281	-0.417	-0.293	0.065	-0.087	0.172	0.138	-0.365	-0.260	-0.186	-0.202	0.132	0.338	**0.743**	-0.374	-0.081	-0.197
AL2	-0.308	-0.384	-0.180	-0.228	-0.303	0.059	0.043	0.261	-0.054	-0.054	-0.012	-0.229	0.196	0.373	0.659	-0.461	0.300	-0.288
AL3	-0.509	-0.104	-0.538	-0.255	0.661	-0.362	0.458	-0.501	**-0.669**	-0.560	-0.513	0.194	0.066	0.181	0.032	0.075	**-0.862**	0.371
E1	-0.070	0.125	0.177	0.148	**0.706**	-0.337	0.119	-0.630	-0.346	-0.137	-0.239	-0.006	-0.153	-0.340	-0.366	0.371	**-0.777**	0.069
E2	0.342	**0.716**	0.384	0.295	-0.193	0.292	**-0.767**	0.452	0.342	0.111	0.279	-0.311	-0.092	-0.193	-0.215	-0.009	0.308	-0.261
E3	0.095	0.237	-0.240	-0.484	0.333	-0.252	-0.061	-0.419	-0.211	-0.520	-0.139	-0.225	0.000	-0.052	-0.258	-0.154	**-0.723**	0.381
K1	0.238	-0.030	**0.805**	**0.687**	0.045	0.341	0.049	-0.350	0.350	**0.711**	0.310	-0.273	-0.405	-0.651	-0.431	0.293	0.074	**-0.792**
K2	-0.520	-0.279	-0.431	-0.529	-0.006	0.050	0.302	-0.072	-0.166	-0.159	0.065	-0.476	-0.102	0.032	0.596	-0.514	-0.220	-0.283
K3	-0.561	-0.419	-0.548	-0.124	0.523	-0.119	**0.683**	-0.424	-0.591	-0.457	-0.357	0.301	0.267	0.282	0.084	0.121	-0.655	0.213
K4	0.224	-0.239	0.237	0.598	0.260	0.218	0.258	-0.081	0.031	0.062	0.228	0.614	0.510	-0.046	-0.577	**0.789**	0.001	0.161
K5	-0.202	-0.399	-0.383	0.184	0.352	-0.007	0.539	-0.146	-0.393	-0.384	-0.228	**0.745**	0.595	0.419	-0.218	0.473	-0.271	0.475
S1	-0.487	-0.197	-0.142	0.151	**0.671**	-0.110	0.588	-0.601	-0.455	-0.077	-0.239	0.088	-0.161	-0.227	-0.101	0.300	**-0.722**	-0.120
S2	0.351	-0.301	-0.023	0.252	0.045	0.216	0.151	0.011	0.011	-0.319	0.146	0.592	**0.830**	0.365	-0.521	0.463	0.008	0.427
S3	0.602	-0.242	0.056	0.065	-0.167	0.143	0.025	-0.037	0.184	-0.252	0.154	0.468	**0.678**	0.322	-0.590	0.269	0.094	0.505

S4	0.357	-0.144	-0.109	0.075	0.145	0.087	0.147	-0.068	-0.002	-0.347	0.153	0.548	0.648	0.194	-0.570	0.477	-0.154	0.590
S5	-0.441	-0.173	0.115	0.345	**0.705**	-0.293	0.584	**-0.673**	-0.406	0.254	-0.323	0.175	-0.456	-0.446	-0.121	0.471	-0.605	-0.176
T1	-0.045	0.041	-0.417	-0.308	0.504	-0.225	0.207	-0.401	-0.397	-0.655	-0.219	0.207	0.312	0.149	-0.331	0.157	**-0.770**	0.593
T2	-0.266	0.057	-0.589	-0.345	0.558	-0.327	0.124	-0.200	-0.593	**-0.845**	-0.372	0.275	0.473	0.395	-0.055	0.107	**-0.741**	0.663
T3	-0.294	0.119	-0.607	-0.314	0.600	-0.398	0.112	-0.162	-0.627	**-0.825**	-0.428	0.368	0.426	0.383	-0.048	0.182	**-0.724**	**0.743**
T4	**-0.667**	-0.162	-0.584	-0.118	**0.709**	-0.345	0.433	-0.294	**-0.776**	-0.622	-0.530	0.332	0.285	0.343	0.199	0.185	**-0.747**	0.354
T5	-0.610	-0.261	-0.503	0.008	0.541	-0.266	0.321	-0.118	**-0.737**	-0.630	-0.551	0.389	0.501	0.577	0.280	0.132	-0.504	0.277
T6	-0.523	-0.492	-0.362	0.154	0.294	-0.103	0.465	-0.117	-0.558	-0.356	-0.482	0.437	0.452	0.586	0.267	0.096	-0.221	0.083
T7	-0.229	0.169	-0.457	-0.397	0.556	-0.162	0.218	-0.352	-0.351	-0.522	-0.042	-0.042	0.047	-0.136	-0.168	0.103	**-0.809**	0.391
T8	-0.288	0.213	-0.584	-0.511	0.583	-0.358	0.151	-0.295	-0.485	**-0.666**	-0.237	0.049	0.082	0.030	-0.046	0.048	**-0.831**	0.592
T9	-0.308	-0.159	-0.647	-0.411	0.577	-0.359	0.362	-0.346	-0.615	**-0.801**	-0.374	0.343	0.464	0.375	-0.047	0.129	**-0.795**	**0.699**
T10	0.087	0.200	-0.358	-0.401	0.404	-0.207	0.081	-0.306	-0.213	-0.528	-0.028	0.100	0.156	-0.059	-0.364	0.149	-0.664	0.616
T11	**-0.813**	-0.075	-0.294	-0.015	0.419	-0.187	0.326	-0.108	-0.481	-0.011	-0.311	-0.130	-0.316	-0.061	0.575	-0.065	-0.329	-0.307
T12	-0.347	-0.142	0.134	0.266	0.320	0.223	0.525	-0.414	-0.005	0.448	0.198	-0.192	-0.501	-0.618	-0.077	0.226	-0.291	-0.555
T13	-0.390	0.221	-0.512	-0.253	**0.765**	-0.495	0.115	-0.251	**-0.667**	**-0.693**	-0.431	0.285	0.195	0.140	-0.025	0.279	**-0.833**	0.642
T14	-0.007	0.492	0.014	0.001	0.423	0.032	-0.127	-0.137	-0.025	-0.089	0.221	-0.189	-0.211	-0.497	-0.319	0.269	-0.466	0.040
T15	**-0.718**	0.391	-0.524	-0.469	0.342	-0.297	-0.066	0.054	-0.452	-0.312	-0.314	-0.419	-0.434	-0.048	0.632	-0.395	-0.454	-0.052
T16	-0.272	0.043	-0.646	-0.583	0.461	-0.334	0.086	-0.133	-0.502	**-0.829**	-0.209	0.080	0.421	0.308	0.105	-0.045	**-0.696**	0.640
T17	**-0.715**	-0.008	-0.483	-0.007	0.665	-0.234	0.486	-0.282	-0.618	-0.273	-0.404	0.232	-0.136	-0.003	0.177	0.229	-0.638	0.137
T18	-0.494	-0.465	**-0.702**	-0.573	0.154	0.019	0.542	-0.273	-0.404	-0.569	-0.165	-0.114	0.301	0.399	0.343	-0.406	-0.513	0.109
T19	-0.236	-0.464	-0.416	0.001	0.394	-0.061	0.432	-0.232	-0.507	-0.644	-0.287	0.496	**0.776**	0.579	-0.095	0.235	-0.463	0.405
T20	-0.550	-0.164	-0.610	-0.021	0.296	-0.295	0.180	0.175	**-0.681**	-0.633	-0.660	0.570	0.522	**0.806**	0.380	0.045	-0.188	0.465
T21	**-0.700**	0.021	-0.373	-0.283	0.303	-0.053	0.276	-0.118	-0.315	-0.058	-0.078	-0.392	-0.388	-0.200	0.521	-0.256	-0.389	-0.319
T22	**-0.822**	-0.098	**-0.776**	-0.475	0.476	-0.410	0.293	-0.106	**-0.765**	**-0.667**	-0.587	0.028	0.112	0.455	0.625	-0.273	-0.613	0.272

A-GF	**1**	-0.100	0.640	0.183	-0.629	0.629	-0.221	-0.076	**0.834**	0.360	**0.702**	-0.251	0.185	-0.247	**-0.769**	-0.050	0.429	-0.273
A-F	-0.100	**1**	-0.083	-0.057	0.064	-0.255	**-0.785**	0.585	-0.056	-0.094	-0.161	-0.142	-0.401	-0.116	0.223	-0.056	0.125	0.198
A-GM	0.640	-0.083	**1**	**0.709**	-0.233	0.369	-0.144	-0.210	0.602	**0.775**	0.455	-0.169	-0.201	-0.561	-0.591	0.322	0.384	-0.646
A-MTU	0.183	-0.057	**0.709**	**1**	0.154	0.126	0.006	-0.016	0.124	0.575	0.040	0.438	0.001	-0.253	-0.406	**0.740**	0.319	-0.330
A-SO	-0.629	0.064	-0.233	0.154	**1**	**-0.721**	0.309	-0.388	**-0.812**	-0.292	-0.629	0.477	-0.080	-0.067	0.107	0.606	**-0.777**	0.418
A-PI	0.629	-0.255	0.369	0.126	**-0.721**	**1**	0.070	0.029	**0.837**	0.405	**0.879**	-0.431	0.137	-0.243	-0.502	-0.250	0.478	-0.627
F/T-SW	-0.221	**-0.785**	-0.144	0.006	0.309	0.070	**1**	**-0.729**	-0.165	0.115	0.033	0.242	0.028	-0.173	-0.211	0.261	-0.458	-0.049
F/T-BT	-0.076	0.585	-0.210	-0.016	-0.388	0.029	**-0.729**	**1**	0.065	-0.149	-0.049	0.071	0.133	0.417	0.475	-0.207	**0.675**	0.168
F/T-GF	**0.834**	-0.056	0.602	0.124	**-0.812**	**0.837**	-0.165	0.065	**1**	0.627	**0.902**	-0.512	-0.134	-0.459	-0.541	-0.242	0.622	-0.602
F/T-MTU	0.360	-0.094	**0.775**	0.575	-0.292	0.405	0.115	-0.149	0.627	**1**	0.501	-0.191	-0.577	**-0.748**	-0.354	0.248	0.473	**-0.717**
F/T-PI	**0.702**	-0.161	0.455	0.040	-0.629	**0.879**	0.033	-0.049	**0.902**	0.501	**1**	-0.512	-0.027	-0.518	-0.558	-0.154	0.392	-0.567
F/T-SO	-0.251	-0.142	-0.169	0.438	0.477	-0.431	0.242	0.071	-0.512	-0.191	-0.512	**1**	0.396	0.377	-0.053	**0.741**	-0.058	0.626
AE-TB	0.185	-0.401	-0.201	0.001	-0.080	0.137	0.028	0.133	-0.134	-0.577	-0.027	0.396	**1**	**0.726**	-0.133	0.064	0.033	0.371
AE-SAL	-0.247	-0.116	-0.561	-0.253	-0.067	-0.243	-0.173	0.417	-0.459	**-0.748**	-0.518	0.377	**0.726**	**1**	0.446	-0.257	0.077	0.527
AE-MD	**-0.769**	0.223	-0.591	-0.406	0.107	-0.502	-0.211	0.475	-0.541	-0.354	-0.558	-0.053	-0.133	0.446	**1**	-0.409	0.027	0.170
AE-SO	-0.050	-0.056	0.322	**0.740**	0.606	-0.250	0.261	-0.207	-0.242	0.248	-0.154	**0.741**	0.064	-0.257	-0.409	**1**	-0.152	0.214
AE-BI	0.429	0.125	0.384	0.319	**-0.777**	0.478	-0.458	**0.675**	0.622	0.473	0.392	-0.058	0.033	0.077	0.027	-0.152	**1**	-0.344
AE-MB	-0.273	0.198	-0.646	-0.330	0.418	-0.627	-0.049	0.168	-0.602	**-0.717**	-0.567	0.626	0.371	0.527	0.170	0.214	-0.344	**1**

Appendix 2 - Correlation matrix (Pearson) for HAD and FD samples

Code	AP1	AP2	AP3	AP4	AP5	AP6	AP7	CP1	TP1	CT2	AT1	AT2	AT3	AT4	FT1	FT2	FT3	FT4	FT5	FT6	FT7	AE1	AE2	AE3	AE4	AE5	AE5
AP1	**1**	0.771	0.427	0.063	0.801	**-0.824**	0.286	-0.501	-0.481	-0.447	0.507	-0.046	0.104	0.367	0.087	**0.953**	0.093	-0.161	0.457	0.320	-0.419	-0.118	0.379	0.261	0.321	0.174	0.174
AP2	0.771	**1**	**0.836**	-0.103	**0.831**	-0.747	-0.331	**-0.845**	**-0.851**	**-0.848**	0.766	0.149	-0.376	0.710	0.359	0.655	0.394	-0.733	0.721	0.665	-0.095	0.352	0.792	0.605	0.513	0.487	0.487
AP3	0.427	**0.836**	**1**	-0.090	0.572	-0.566	-0.661	**-0.927**	**-0.939**	-0.765	**0.812**	-0.080	-0.663	0.756	0.099	0.369	0.142	**-0.895**	0.436	0.444	-0.138	0.495	0.726	0.483	0.262	0.234	0.234
AP4	0.063	-0.103	-0.090	**1**	-0.465	0.448	-0.138	0.306	0.262	0.289	0.151	0.013	0.305	-0.345	-0.248	0.323	-0.296	0.259	-0.120	-0.350	0.056	-0.805	-0.271	-0.680	-0.587	-0.334	-0.334
AP5	0.801	**0.831**	0.572	-0.465	**1**	**-0.929**	0.093	-0.773	-0.745	-0.764	0.374	0.161	-0.335	0.735	0.407	0.594	0.436	-0.505	0.660	0.672	-0.174	0.486	0.715	0.762	0.749	0.527	0.527
AP6	**-0.824**	-0.747	-0.566	0.448	**-0.929**	**1**	-0.219	0.713	0.671	0.543	-0.462	0.212	0.193	-0.571	-0.043	-0.676	-0.077	0.363	-0.349	-0.360	0.522	-0.358	-0.470	-0.536	-0.482	-0.181	-0.181
AP7	0.286	-0.331	-0.661	-0.138	0.093	-0.219	**1**	0.491	0.542	0.516	-0.468	-0.231	0.694	-0.465	-0.246	0.281	-0.271	0.803	-0.315	-0.353	-0.437	-0.426	-0.542	-0.259	-0.049	-0.283	-0.283
CP1	-0.501	**-0.845**	**-0.927**	0.306	-0.773	0.713	0.491	**1**	**0.998**	**0.881**	-0.587	-0.089	0.767	**-0.927**	-0.293	-0.358	-0.331	**0.883**	-0.577	-0.621	0.054	-0.659	**-0.853**	-0.716	-0.553	-0.424	-0.424
TP1	-0.481	**-0.851**	**-0.939**	0.262	-0.745	0.671	0.542	**0.998**	**1**	**0.895**	-0.603	-0.124	0.780	**-0.928**	-0.316	-0.347	-0.353	**0.906**	-0.600	-0.637	0.009	-0.647	**-0.871**	-0.708	-0.543	-0.443	-0.443
CT2	-0.447	**-0.848**	-0.765	0.289	-0.764	0.543	0.516	**0.881**	**0.895**	**1**	-0.433	-0.517	0.722	**-0.923**	-0.697	-0.250	-0.726	**0.885**	**-0.883**	**-0.911**	-0.345	-0.674	**-0.994**	**-0.872**	-0.791	-0.791	-0.791
AT1	0.507	0.766	**0.812**	0.151	0.374	-0.462	-0.468	-0.587	-0.603	-0.433	**1**	-0.336	-0.124	0.277	-0.173	0.547	-0.128	-0.582	0.218	0.145	-0.389	0.066	0.357	0.092	-0.109	-0.043	-0.043
AT2	-0.046	0.149	-0.080	0.013	0.161	0.212	-0.231	-0.089	-0.124	-0.517	-0.336	**1**	-0.326	0.393	**0.943**	-0.188	**0.918**	-0.267	0.787	0.771	**0.915**	0.250	0.583	0.536	0.685	**0.876**	**0.876**
AT3	0.104	-0.376	-0.663	0.305	-0.335	0.193	0.694	0.767	0.780	0.722	-0.124	-0.326	**1**	**-0.872**	-0.404	0.237	-0.424	**0.833**	-0.435	-0.557	-0.379	-0.782	-0.761	-0.662	-0.508	-0.458	-0.458

AT4	0.367	0.710	0.756	-0.345	0.735	-0.571	-0.465	**-0.927**	**-0.928**	**-0.923**	0.277	0.393	**-0.872**	**1**	0.544	0.181	0.566	**-0.844**	0.706	0.769	0.246	0.733	**0.931**	**0.836**	0.745	0.631	0.631
FT1	0.087	0.359	0.099	-0.248	0.407	-0.043	-0.246	-0.293	-0.316	-0.697	-0.173	**0.943**	-0.404	0.544	**1**	-0.128	**0.997**	-0.442	**0.895**	**0.922**	**0.811**	0.478	0.740	0.763	**0.858**	**0.985**	**0.985**
FT2	**0.953**	0.655	0.369	0.323	0.594	-0.676	0.281	-0.358	-0.347	-0.250	0.547	-0.188	0.237	0.181	-0.128	**1**	-0.133	-0.025	0.269	0.082	-0.513	-0.369	0.183	-0.025	0.038	-0.063	-0.063
FT3	0.093	0.394	0.142	-0.296	0.436	-0.077	-0.271	-0.331	-0.353	-0.726	-0.128	**0.918**	-0.424	0.566	**0.997**	-0.133	**1**	-0.485	**0.901**	**0.940**	0.789	0.526	0.763	0.797	**0.872**	**0.994**	**0.994**
FT4	-0.161	-0.733	**-0.895**	0.259	-0.505	0.363	0.803	**0.883**	**0.906**	**0.885**	-0.582	-0.267	**0.833**	**-0.844**	-0.442	-0.025	-0.485	**1**	-0.621	-0.692	-0.266	-0.736	**-0.879**	-0.711	-0.505	-0.550	-0.550
FT5	0.457	0.721	0.436	-0.120	0.660	-0.349	-0.315	-0.577	-0.600	**-0.883**	0.218	0.787	-0.435	0.706	**0.895**	0.269	**0.901**	-0.621	**1**	**0.964**	0.564	0.419	**0.892**	0.781	**0.832**	**0.931**	**0.931**
FT6	0.320	0.665	0.444	-0.350	0.672	-0.360	-0.353	-0.621	-0.637	**-0.911**	0.145	0.771	-0.557	0.769	**0.922**	0.082	**0.940**	-0.692	**0.964**	**1**	0.590	0.633	**0.924**	**0.906**	**0.917**	**0.971**	**0.971**
FT7	-0.419	-0.095	-0.138	0.056	-0.174	0.522	-0.437	0.054	0.009	-0.345	-0.389	**0.915**	-0.379	0.246	**0.811**	-0.513	0.789	-0.266	0.564	0.590	**1**	0.269	0.429	0.374	0.459	0.725	0.725
AE1	-0.118	0.352	0.495	-0.805	0.486	-0.358	-0.426	-0.659	-0.647	-0.674	0.066	0.250	-0.782	0.733	0.478	-0.369	0.526	-0.736	0.419	0.633	0.269	**1**	0.682	**0.863**	0.697	0.570	0.570
AE2	0.379	0.792	0.726	-0.271	0.715	-0.470	-0.542	**-0.853**	**-0.871**	**-0.994**	0.357	0.583	-0.761	**0.931**	0.740	0.183	0.763	**-0.879**	**0.892**	**0.924**	0.429	0.682	**1**	**0.874**	0.805	**0.819**	**0.819**
AE3	0.261	0.605	0.483	-0.680	0.762	-0.536	-0.259	-0.716	-0.708	**-0.872**	0.092	0.536	-0.662	**0.836**	0.763	-0.025	0.797	-0.711	0.781	**0.906**	0.374	**0.863**	**0.874**	**1**	**0.949**	**0.847**	**0.847**
AE4	0.321	0.513	0.262	-0.587	0.749	-0.482	-0.049	-0.553	-0.543	-0.791	-0.109	0.685	-0.508	0.745	**0.858**	0.038	**0.872**	-0.505	**0.832**	**0.917**	0.459	0.697	0.805	**0.949**	**1**	**0.904**	**0.904**
AE5	0.174	0.487	0.234	-0.334	0.527	-0.181	-0.283	-0.424	-0.443	-0.791	-0.043	**0.876**	-0.458	0.631	**0.985**	-0.063	**0.994**	-0.550	**0.931**	**0.971**	0.725	0.570	**0.819**	**0.847**	**0.904**	**1**	**1**
T1	0.594	0.093	-0.025	0.444	0.088	-0.353	0.527	0.117	0.141	0.387	0.298	-0.636	0.566	-0.341	-0.679	0.772	-0.696	0.462	-0.394	-0.565	-0.806	-0.699	-0.450	-0.577	-0.515	-0.659	-0.659
T2	-0.587	-0.590	-0.637	0.162	-0.491	0.733	0.008	0.521	0.494	0.187	-0.800	0.705	-0.017	-0.180	0.487	-0.600	0.439	0.296	0.118	0.130	**0.818**	-0.088	-0.090	-0.037	0.150	0.337	0.337

T3	0.425	-0.143	-0.277	0.506	-0.120	-0.138	0.629	0.373	0.396	0.591	0.100	-0.598	0.721	-0.552	-0.700	0.628	-0.728	0.671	-0.512	-0.685	-0.723	**0.831**	-0.639	-0.725	-0.616	-0.720	-0.720
T4	0.427	-0.065	-0.058	0.157	0.080	-0.407	0.590	0.088	0.132	0.464	0.142	-0.799	0.434	-0.298	-0.805	0.565	**-0.812**	0.474	-0.593	-0.671	**-0.918**	-0.483	-0.523	-0.513	-0.499	-0.771	-0.771
T5	-0.081	-0.515	-0.673	0.770	-0.554	0.561	0.394	0.726	0.707	0.601	-0.432	0.137	0.604	-0.619	-0.160	0.121	-0.227	0.741	-0.252	-0.439	0.133	**-0.884**	-0.561	-0.708	-0.466	-0.306	-0.306
T6	0.399	0.257	0.443	0.103	0.163	-0.491	0.071	-0.291	-0.264	0.152	0.607	**-0.885**	0.099	-0.035	-0.799	0.527	-0.774	-0.030	-0.471	-0.512	**-0.915**	-0.187	-0.228	-0.339	-0.498	-0.698	-0.698
T7	-0.434	-0.372	-0.482	0.395	-0.461	0.741	-0.157	0.456	0.409	0.061	-0.531	0.786	0.036	-0.169	0.563	-0.412	0.515	0.174	0.295	0.234	**0.875**	-0.215	0.024	-0.068	0.113	0.420	0.420
T8	0.387	0.376	0.531	-0.129	0.273	-0.573	-0.007	-0.374	-0.346	0.020	0.718	**-0.855**	0.065	0.021	-0.676	0.446	-0.629	-0.180	-0.360	-0.352	**-0.879**	0.024	-0.111	-0.144	-0.354	-0.540	-0.540
T9	0.410	0.292	0.523	-0.031	0.349	-0.599	0.032	-0.527	-0.494	-0.084	0.375	-0.646	-0.283	0.340	-0.576	0.467	-0.563	-0.188	-0.287	-0.287	-0.725	0.069	0.041	-0.057	-0.185	-0.485	-0.485
T10	-0.681	-0.595	-0.547	0.184	-0.559	0.792	-0.146	0.468	0.435	0.166	-0.741	0.670	-0.125	-0.141	0.447	-0.679	0.403	0.198	0.081	0.108	**0.841**	-0.032	-0.065	-0.050	0.091	0.301	0.301
T11	-0.036	-0.106	-0.266	0.239	0.020	0.260	0.016	0.053	0.031	-0.237	-0.583	**0.832**	-0.257	0.287	0.653	-0.087	0.597	0.040	0.497	0.437	0.734	-0.032	0.321	0.235	0.457	0.541	0.541
T12	0.038	0.068	-0.092	0.575	-0.085	0.369	-0.237	0.044	-0.004	-0.277	-0.227	0.811	-0.158	0.196	0.608	0.068	0.556	-0.074	0.566	0.423	0.747	-0.237	0.346	0.069	0.251	0.504	0.504
T13	-0.345	-0.173	-0.240	0.403	-0.321	0.634	-0.350	0.211	0.158	-0.168	-0.389	**0.858**	-0.191	0.073	0.658	-0.342	0.615	-0.068	0.458	0.396	**0.930**	-0.079	0.255	0.084	0.227	0.537	0.537
T14	0.303	0.498	0.719	-0.134	0.279	-0.512	-0.308	-0.528	-0.516	-0.180	**0.840**	-0.714	-0.151	0.184	-0.518	0.341	-0.462	-0.443	-0.197	-0.171	-0.688	0.196	0.099	0.000	-0.264	-0.372	-0.372
T15	-0.137	0.018	0.229	-0.477	-0.014	-0.231	-0.052	-0.081	-0.053	0.188	0.423	-0.749	0.101	-0.196	-0.541	-0.149	-0.479	-0.107	-0.483	-0.347	-0.611	0.270	-0.257	-0.068	-0.308	-0.429	-0.429
T16	-0.611	-0.466	-0.483	0.140	-0.458	0.739	-0.197	0.401	0.363	0.034	-0.664	0.779	-0.142	-0.073	0.592	-0.643	0.553	0.100	0.242	0.266	**0.919**	0.035	0.062	0.074	0.215	0.458	0.458
T17	-0.191	-0.429	-0.720	0.066	-0.166	0.398	0.404	0.502	0.496	0.145	**-0.821**	0.712	0.204	-0.168	0.549	-0.253	0.496	0.460	0.248	0.220	0.633	-0.185	-0.074	0.060	0.339	0.414	0.414

T18	0.088	0.349	0.719	-0.233	0.199	-0.425	-0.406	-0.580	-0.565	-0.184	0.655	-0.686	-0.399	0.307	-0.518	0.110	-0.465	-0.508	-0.276	-0.190	-0.589	0.370	0.130	0.060	-0.229	-0.384	-0.384
T19	0.053	0.313	0.689	-0.324	0.219	-0.441	-0.373	-0.585	-0.565	-0.188	0.580	-0.671	-0.438	0.335	-0.493	0.052	-0.440	-0.503	-0.283	-0.172	-0.574	0.440	0.137	0.113	-0.173	-0.360	-0.360
T20	0.104	0.311	0.641	-0.201	0.145	-0.405	-0.318	-0.466	-0.449	-0.069	0.690	-0.777	-0.225	0.152	-0.606	0.148	-0.552	-0.396	-0.359	-0.288	-0.680	0.256	0.002	-0.049	-0.332	-0.473	-0.473
T21	0.161	-0.342	-0.302	0.413	-0.313	-0.012	0.549	0.434	0.460	0.745	0.043	-0.788	0.652	-0.634	**-0.894**	0.386	**-0.914**	0.684	-0.770	**-0.881**	-0.794	-0.746	-0.788	**-0.823**	-0.783	**-0.912**	**-0.912**
T22	-0.665	-0.626	-0.606	0.305	-0.637	**0.853**	-0.109	0.570	0.533	0.260	-0.706	0.629	0.012	-0.269	0.381	-0.625	0.333	0.290	0.029	0.023	0.803	-0.181	-0.164	-0.179	-0.023	0.226	0.226
T23	-0.477	-0.472	-0.648	-0.018	-0.326	0.602	0.099	0.499	0.478	0.090	-0.759	0.778	0.066	-0.166	0.636	-0.557	0.598	0.271	0.264	0.291	**0.829**	0.006	-0.009	0.128	0.326	0.506	0.506
T24	0.083	0.201	-0.108	-0.236	0.365	-0.013	-0.024	-0.145	-0.159	-0.552	-0.388	**0.949**	-0.291	0.446	**0.964**	-0.125	**0.947**	-0.227	0.808	**0.827**	0.787	0.366	0.603	0.681	**0.844**	**0.921**	**0.921**
T25	0.410	0.590	**0.830**	0.053	0.479	-0.536	-0.439	**-0.840**	**-0.839**	-0.560	0.503	-0.168	-0.696	0.744	-0.102	0.411	-0.088	-0.648	0.209	0.197	-0.245	0.322	0.546	0.289	0.141	-0.011	-0.011
T26	0.010	0.244	0.502	-0.625	0.475	-0.485	-0.233	-0.700	-0.670	-0.490	-0.018	-0.049	**-0.813**	0.753	0.101	-0.154	0.129	-0.557	0.125	0.302	-0.064	0.796	0.500	0.615	0.471	0.184	0.184
T27	-0.386	-0.060	-0.029	-0.139	-0.011	0.339	-0.417	-0.146	-0.177	-0.455	-0.464	**0.873**	-0.616	0.476	0.802	-0.527	0.784	-0.370	0.560	0.638	**0.931**	0.483	0.544	0.540	0.602	0.735	0.735
T28	0.400	0.636	0.410	-0.451	0.780	-0.504	-0.181	-0.665	-0.664	**-0.889**	0.030	0.704	-0.588	**0.835**	**0.863**	0.143	**0.876**	-0.619	**0.907**	**0.959**	0.471	0.666	**0.902**	**0.943**	**0.977**	**0.915**	**0.915**
S1	0.578	0.806	0.589	-0.129	0.781	-0.525	-0.310	-0.755	-0.769	**-0.940**	0.283	0.651	-0.569	**0.856**	0.769	0.399	0.776	-0.690	**0.951**	**0.916**	0.396	0.461	**0.943**	0.798	**0.824**	**0.826**	**0.826**
S2	-0.416	-0.351	-0.031	-0.666	-0.105	-0.111	0.104	-0.005	0.039	0.289	-0.149	-0.606	-0.148	-0.077	-0.477	-0.473	-0.438	0.038	-0.608	-0.390	-0.434	0.442	-0.305	0.016	-0.158	-0.418	-0.418
S3	-0.404	-0.390	-0.550	0.037	-0.247	0.545	0.040	0.373	0.350	-0.022	-0.750	**0.848**	-0.070	-0.014	0.690	-0.485	0.647	0.183	0.354	0.367	**0.871**	0.035	0.111	0.191	0.396	0.560	0.560
AL1	-0.244	-0.142	-0.344	-0.326	0.106	0.215	0.037	0.078	0.070	-0.306	-0.658	**0.879**	-0.266	0.273	**0.851**	-0.439	**0.830**	-0.054	0.551	0.629	**0.825**	0.383	0.378	0.557	0.719	0.776	0.776

AL2	0.136	0.240	0.562	-0.274	0.371	-0.478	-0.215	-0.676	-0.650	-0.336	0.123	-0.279	-0.711	0.651	-0.206	0.091	-0.192	-0.447	-0.063	0.031	-0.297	0.480	0.338	0.299	0.157	-0.132	-0.132
AL3	0.071	-0.012	-0.368	-0.260	0.296	0.000	0.285	0.066	0.068	-0.309	-0.610	**0.841**	-0.088	0.257	**0.824**	-0.121	0.796	0.072	0.613	0.631	0.654	0.216	0.361	0.533	0.758	0.757	0.757
AL4	-0.003	-0.005	0.367	-0.329	0.140	-0.401	-0.016	-0.388	-0.348	0.062	0.148	-0.699	-0.367	0.245	-0.607	0.011	-0.581	-0.161	-0.493	-0.376	-0.636	0.308	-0.088	-0.019	-0.203	-0.524	-0.524
AL5	-0.375	-0.337	-0.337	-0.562	-0.214	0.096	0.247	0.394	0.420	0.400	-0.073	-0.380	0.408	-0.502	-0.231	-0.441	-0.189	0.261	-0.417	-0.268	-0.244	0.166	-0.438	-0.070	-0.159	-0.196	-0.196
E1	0.198	0.203	0.426	-0.085	0.364	-0.389	-0.135	-0.599	-0.581	-0.360	-0.063	-0.017	-0.692	0.678	-0.028	0.163	-0.039	-0.346	0.089	0.126	-0.094	0.334	0.387	0.289	0.251	0.001	0.001
E2	-0.595	-0.501	-0.098	-0.542	-0.278	0.095	0.001	0.062	0.096	0.337	-0.296	-0.502	-0.249	-0.076	-0.450	-0.621	-0.425	0.054	-0.643	-0.427	-0.267	0.411	-0.326	-0.054	-0.212	-0.428	-0.428
E3	0.052	0.317	0.104	-0.217	0.383	-0.018	-0.268	-0.317	-0.341	-0.697	-0.237	**0.958**	-0.488	0.596	**0.988**	-0.155	**0.979**	-0.449	**0.873**	**0.903**	**0.838**	0.488	0.751	0.755	**0.854**	**0.963**	**0.963**
E4	0.633	0.786	0.718	0.483	0.360	-0.354	-0.434	-0.526	-0.557	-0.483	**0.880**	-0.039	-0.093	0.312	0.014	0.711	0.028	-0.497	0.419	0.250	-0.166	-0.172	0.432	0.034	-0.051	0.092	0.092
K1	-0.219	-0.565	**-0.862**	-0.043	-0.215	0.364	0.612	0.642	0.652	0.349	**-0.882**	0.499	0.384	-0.350	0.357	-0.274	0.309	0.650	0.031	0.025	0.427	-0.244	-0.293	-0.056	0.223	0.228	0.228
K2	-0.358	-0.758	-0.774	-0.339	-0.350	0.174	0.749	0.671	0.719	0.766	-0.655	-0.400	0.529	-0.628	-0.433	-0.349	-0.443	0.785	-0.700	-0.603	-0.339	-0.195	-0.765	-0.372	-0.281	-0.485	-0.485
K3	0.012	-0.274	-0.240	-0.625	0.137	-0.389	0.628	0.121	0.187	0.395	-0.212	-0.652	0.247	-0.220	-0.511	-0.050	-0.487	0.378	-0.568	-0.431	-0.686	0.145	-0.439	-0.054	-0.094	-0.456	-0.456
ALA1	-0.247	-0.453	-0.739	0.048	-0.209	0.447	0.376	0.539	0.530	0.163	**-0.815**	0.717	0.225	-0.209	0.559	-0.312	0.509	0.458	0.239	0.219	0.661	-0.172	-0.092	0.054	0.324	0.423	0.423
ALA2	-0.201	-0.685	**-0.915**	-0.191	-0.241	0.213	**0.851**	0.723	0.759	0.621	**-0.831**	0.031	0.575	-0.550	-0.060	-0.224	-0.095	**0.845**	-0.355	-0.326	-0.025	-0.306	-0.597	-0.249	-0.022	-0.158	-0.158
ALE1	**-0.849**	-0.707	-0.403	-0.502	-0.520	0.448	-0.004	0.385	0.402	0.446	-0.504	-0.214	-0.067	-0.311	-0.210	**-0.889**	-0.193	0.195	-0.563	-0.346	0.082	0.337	-0.412	-0.088	-0.183	-0.240	-0.240
ALE2	-0.806	-0.613	-0.307	-0.554	-0.462	0.378	-0.058	0.315	0.332	0.390	-0.375	-0.269	-0.074	-0.286	-0.223	**-0.857**	-0.195	0.113	-0.540	-0.315	0.027	0.392	-0.369	-0.044	-0.175	-0.229	-0.229

UK 1	0.391	0.226	-0.292	0.218	0.219	0.011	0.284	0.221	0.202	-0.180	-0.088	0.637	0.420	-0.104	0.610	0.331	0.585	0.189	0.604	0.462	0.405	-0.307	0.180	0.153	0.379	0.560	0.560
UK 2	-0.152	-0.075	-0.279	-0.237	0.162	0.166	0.019	0.002	-0.008	-0.367	-0.633	**0.911**	-0.323	0.357	**0.863**	-0.335	**0.835**	-0.089	0.606	0.658	**0.824**	0.347	0.442	0.562	0.737	0.786	0.786
UK 3	-0.571	-0.266	0.177	-0.601	-0.125	0.030	-0.301	-0.222	-0.201	0.007	-0.153	-0.303	-0.560	0.231	-0.211	-0.650	-0.176	-0.295	-0.368	-0.132	-0.067	0.665	0.013	0.216	0.007	-0.165	-0.165

Numbers in bold denote significant correlations

Appendix 3 – Microbial analysis report at the beginning of the trial (M1)

SYNLAB

UKAS TESTING 4412

CERTIFICATE OF ANALYSIS

FAO:	Dr Sameer Khalil Ghawi	Order Number:	3290993
Company:	University of Reading	Date Received:	17/03/2021
Address:	Department of Food and Nutritional Sciences University of Reading Whiteknights Reading	Analysis Started	17/03/2021
		Report Date:	22/03/2021
Reference:		Report Number:	21-14743

Lab No	Client Reference	Sample Description	Specification
2239199	MG FD ST1	MANGO GINGER (SPICE POWDER)	
2239200	MG TD ST1	MANGO GINGER (SPICE POWDER)	

Lab No.	Test Ref	Analysis	Result	Units
2239199	MIC1004	Aerobic Colony Count 72h at 30ºC	4.10x10^3	cfu/g
2239199	MIC1005	Osmophilic Moulds	< 20	cfu/g
2239199	MIC1005	Osmophilic Yeasts	< 20	cfu/g
2239199	MIC1018	Enterobacteriaceae (presumptive)	320	cfu/g
2239199	MIC1022	ß-glucuronidase positive E.coli	< 10	cfu/g
2239199	MIC1026	Bacillus cereus (presumptive)	< 20	cfu/g
2239199	MIC1027	Clostridium perfringens (presumpt)	< 10	cfu/g
2239199	MIC1023	Salmonella spp. (detection)	Not Detected	in 25g
2239200	MIC1004	Aerobic Colony Count 72h at 30ºC	1.73x10^4	cfu/g
2239200	MIC1005	Osmophilic Moulds	< 20	cfu/g
2239200	MIC1005	Osmophilic Yeasts	< 20	cfu/g
2239200	MIC1018	Enterobacteriaceae (presumptive)	> 1.50x10^3	cfu/g
2239200	MIC1022	ß-glucuronidase positive E.coli	< 10	cfu/g
2239200	MIC1026	Bacillus cereus (presumptive)	< 20	cfu/g
2239200	MIC1027	Clostridium perfringens (presumpt)	< 10	cfu/g
2239200	MIC1023	Salmonella spp. (detection)	Not Detected	in 25g

cfu = colony forming units

Appendix 4 – Microbial analysis report at the end of the trial (M3)

UKAS TESTING 4412

CERTIFICATE OF ANALYSIS

FAO: Dr Sameer Khalil Ghawi
Company: University of Reading
Address: Department of Food and Nutritional Sciences
University of Reading
Whiteknights
Reading

Order Number: 3294896
Date Received: 01/07/2021
Analysis Started 01/07/2021
Report Date: 06/07/2021

Reference:

Report Number: 21-28065

Lab No	Client Reference	Sample Description	Specification
2380451	MG TD1	Mango Ginger (Spice Powder)	
2380452	MG FD1	Mango Ginger (Spice Powder)	

Lab No.	Test Ref	Analysis	Result	Units
2380451	MIC1004	Aerobic Colony Count 72h at 30ºC	> 3.00x10^4	cfu/g
2380451	MIC1005	Osmophilic Moulds	< 20	cfu/g
2380451	MIC1005	Osmophilic Yeasts	< 20	cfu/g
2380451	MIC1018	Enterobacteriaceae (presumptive)	> 1.50x10^3	cfu/g
2380451	MIC1022	ß-glucuronidase positive E.coli	< 10	cfu/g
2380451	MIC1026	Bacillus cereus (presumptive)	< 20	cfu/g
2380451	MIC1027	Clostridium perfringens (presumpt)	< 10	cfu/g
2380451	MIC1023	Salmonella spp. (detection)	Not Detected	in 25g
2380452	MIC1004	Aerobic Colony Count 72h at 30ºC	64	cfu/g
2380452	MIC1005	Osmophilic Moulds	20	cfu/g
2380452	MIC1005	Osmophilic Yeasts	< 20	cfu/g
2380452	MIC1018	Enterobacteriaceae (presumptive)	10	cfu/g
2380452	MIC1022	ß-glucuronidase positive E.coli	< 10	cfu/g
2380452	MIC1026	Bacillus cereus (presumptive)	< 20	cfu/g
2380452	MIC1027	Clostridium perfringens (presumpt)	< 10	cfu/g
2380452	MIC1023	Salmonella spp. (detection)	Not Detected	in 25g

cfu = colony forming units

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Tests marked with * do not form part of our current ISO 17025 Scope of Accreditation.
The test results published in this report relate only to the stated sample

Any limits applied to results are as agreed with the customer.

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Tests marked with ‡ or a @ are subcontracted to an ISO 17025 accredited
Results marked with an "E" are estimated counts.