BIOCHEMICAL CHARACTERIZATION OF SOME *CITRUS* GERMPLASM OF ASSAM

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IN

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By

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Dedicated to
my
beloved parents
Dr. Anjan Saikia
&
Mrs. Nibedita Saikia
& sister Abhilasha

ASSAM AGRICULTURAL UNIVERSITY
Faculty of Agriculture

CERTIFICATE – I

This is to certify that the thesis entitled **"Biochemical characterization of some *Citrus* germplasm of Assam"** submitted to the Faculty of Agriculture, Assam Agricultural University, Jorhat-13 in partial fulfilment for the degree of **Master of Science (Agriculture)** in **Horticulture** is a record of research work carried out by **Kareena Saikia, Roll No. 2021-AMJ-78** under my personal supervision and guidance. All kinds of help received by her have been duly acknowledged. No part of this thesis has been reproduced elsewhere for any degree. The thesis is plagiarism free/with similarity content below the accepted norms of A.A.U., Jorhat which does not affect the originality of the research work.

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CERTIFICATE – II

This is to certify that the thesis entitled **"Biochemical characterization of some *Citrus* germplasm of Assam"** submitted by **Kareena Saikia, Roll No. 2021-AMJ-78** to Assam Agricultural University in partial fulfilment of the requirement for the degree of **Master of Science (Agriculture)** in the discipline of **Horticulture** has been examined and approved by the student's Advisory Committee after viva- voce.

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Date: 10 August, 2023 Author

ABSTRACT

Ten genotypes representing each of the five groups of Citrus were collected from AAU-Citrus and Plantation Crops Research Station, Tinsukia and Regional Research Centre for Citrus (CCRI, ICAR), Biswanath Chariali. Morphological characterization of fruits was carried out as per the descriptors developed for Citrus by IPGRI (1999), Rome, Italy and the results indicate the presence of significant variability among genotypes. Biochemical characterization revealed that moisture content ranged from 92.51% to 84.30% in the pulps and from 83.32% to 70.20% in the peels. Juice pH, TSS and titratable acidity ranged from 3.90 to 2.60, 11.40 to 5.30 °Brix, and 0.97% to 4.71% respectively in different genotypes. The ash content varied from 2.83% to 5.96% in the pulps and from 2.53% to 5.69% in the peels. Similarly, the reducing sugar in the juice varied from 2.06% to 6.72% and non-reducing sugar varied from 0.48% to 4.15%. The total sugar percentage ranged from 9.36%-2.54% in the juice. The crude protein content was found to be high and ranged from 5.60%-9.16% in the pulps and 5.60%-13.83% in the peels. Organic acid and water-soluble vitamins were quantified using Ultra High-Performance Liquid Chromatography (UHPLC) and it was revealed that citric acid was present in the maximum amount followed by malic acid. Among the water-soluble vitamins, the thiamine concentration was found to be the highest. Quantification of the minerals was carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results show that both major and minor minerals were present in significant amounts in both the peel and pulp of the *Citrus* species. The essential oil content (%) was estimated and it was found to range from 0.26% to 0.83% in the fresh peel of the *Citrus* genotypes. Analysis of the essential oil that was carried out by Triple Quadrupole Gas-Chromatograph Mass-Spectroscopy (TQGC-MS/MS) showed the presence of D-limonene, α-pinene, ß-pinene, γ -terpinene, α-terpineol, terpinene-4-ol, linalool as the major compounds. Aromatherapeutic, antioxidant, antifungal and insect-repellent compounds such as eucalyptol, thymol, geraniol and nerol were also reported in certain species.

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LIST OF ABBREVIATIONS

%	:	Per cent
/	:	Per
µ	:	Micro
AAU	:	Assam Agricultural University
A.O.A.C	:	Association of Official Analytical Chemist
BNCA	:	Biswananth College of Agriculture
°C	:	Degree Centigrade
cm	:	centimetre
CD	:	Critical Difference
Conc.	:	Concentration
et al.	:	et alia (and other)
CCRI	:	Central Citrus Research Institute
$CuSO_4$	:	Copper Sulphate
DNS	:	Dinitro salicylic Acid
g	:	Gram
GCMS	:	Gas Chromatography Mass Spectroscopy
HCl	:	Hydrochloric acid
HNO_3	:	Nitric acid
HPLC	:	High-performance Liquid Chromatography
H_3PO_4	:	Phosphoric acid
H_2SO_4	:	Sulphuric acid
ICAR	:	India Council for Agricultural Research
ICP-MS	:	Inductively Coupled Plasma Mass Spectroscopy

IPGRI	:	International Plant Genetic Resource Institute
KOH	:	Potassium hydroxide
K_2SO_4	:	Potassium Sulphate
kg	:	Kilogram
M	:	Molar
m	:	Metre
mg	:	Milligram
min	:	Minute
ml	:	millilitre
mm	:	millimetre
nm	:	Nanometer
N	:	Normality
NaOH	:	Sodium Hydroxide
nm	:	nanometre
OD	:	Optical Density
pH	:	Power of Hydrogen
ppm	:	Parts per million
rpm	:	Revolutions per minute
S. ED	:	Standard error deviation
TQGCMS	:	Triple Quadruple Gas Chromatography Mass Spectroscopy
TSS	:	Total Soluble Solids
UHPLC	:	Ultra High-Performance Liquid Chromatography
v	:	volume
α	:	Alpha
β	:	Beta

Introduction…

CHAPTER I

INTRODUCTION

Citrus is one of the most important fruit crops and has tremendous economic and social significance in Indian society. They are commercially grown in 140 countries of the world. The *Citrus* industry makes a significant contribution to the global economy and employs millions of people worldwide in harvesting, handling, shipping, storage, and marketing operations. The *Citrus* genus is a member of the Citrineae subtribe, the Citreae tribe, the Aurantioideae subfamily, and the Rutaceae family (Webber, 1967). Poncirus and Fortunella are the two other important genera in this family. All edible species occur in the subgenus Eucitrus which is classified into five horticultural groups according to Hodgson (1965) namely acid group, mandarin group, orange group, grapefruit and pummelo group, and others species group.

Citrus fruits thrive in tropical and subtropical regions of the world (Hore and Barua, 2004). They require moderate rainfall and are best suited to loam or sandy loam soils (Ladaniya, 2008). *Citrus* fruits should never be grown on saline or alkaline soils or soils with lime nodules. Well-drained soils are essential as the crop does not tolerate waterlogging. The rich genetic variety, notably in mandarins, sweet oranges, lemons, and limes, has greatly aided India's *Citrus* production and industry development. *Citrus* fruits are identified by a leathery rind or peel and a white albedo that encloses the pulp. The pulp is separated into discrete segments or juice sacs containing seeds in the inside section. The seeds are without endosperm. The non-edible components are the peels and seeds. All *Citrus* fruits are a special type of berry called the hesperidium and range in colour from yellow to russet.

Apart from being important to the diet, *Citrus* fruits are also an important item in many Hindu customs. Sanskrit literature contains the earliest known reference of *Citrus* as it appears in the Vajasaneyi Samhita, (a collection of devotional literature dating before 800 B.C.) which forms a part of the Yajur Veda. The seeds, peels, pulp, juice and leaves were used to make Ayurvedic medicines and treat different ailments. The oldest Chinese reference to *Citrus* may be found in the book "Tribute of Yu." written in 776 B.C (Scora *et al.*, 1975).

Citrus fruits have been used for dietary and medicinal purposes and their therapeutic benefits are well known. They are high in carotenoids, flavonoids, terpenes, limonoids,

vitamins, minerals and a range of other bioactive substances having nutritional and nutraceutical significance (Liu *et al.*, 2012). They are gaining popularity for their anti-oxidant, nutritive, insecticidal, antimicrobial and other benefits. *Citrus* plant parts have a high concentration of essential oils as well as other beneficial elements such as vitamin B_9, Vitamin C, potassium, thiamine, manganese, and copper. The fruits are an excellent source of Vitamin C and can be used to cure scurvy (Pope *et al*., 2023). The essential oils are a mixture of over 200 different compounds, the majority of which are monoterpene and sesquiterpene hydrocarbons as well as their oxygenated derivatives such as alcohols, esters, ketones and aliphatic aldehydes (Bakkali *et al.*, 2008). Essential oils were found to have germicidal, antioxidant, and anti-carcinogenic properties. *Citrus* essential oils offer a range of benefits for health and wellness. They are used for aromatherapy, as anti-fungal agents, in air fresheners, massage oils, perfumes and skin care products (Mukhopadhyay, 2000).

Citriculture has evolved into a global industry. *Citrus* ranks second in terms of area (1.095 million ha) and ranks third in terms of production (14.810 million metric tonnes) of fruit crops in India (Agricultural Statistics at a Glance, 2022). *Citrus* crops occupy about 15.46% of the total area under fruit and contribute to 13.81 % of the total production of fruits in India (Agricultural Statistics at a Glance, 2022). According to the Observatory of Economic Complexity, (OEC) Global trade in *Citrus* was about 16.7 billion dollars in 2021. Besides being eaten as fresh fruit and juice, *Citrus* fruits are also processed into jam, jelly, marmalade, candies, *Citrus* wine and brandies. Seed oil, peel oil, citric acid and pectin can be extracted and sold in various forms. *Citrus* fruits can also be used as additives, spices, cosmetics, beverages, and pharmaceutical sectors. It has therapeutic properties for appetite loss, stomach illnesses, gastrointestinal disorders, vitamin deficiencies, nutritional anemia, cardiovascular diseases, and a variety of other immunodeficient conditions. Hesperidin, a flavonoid found in *Citrus* peels, has been discovered as a potentially useful chemical in the battle against COVID-19. Its antiviral effectiveness has been demonstrated for other viruses, particularly SARS-CoV, and it may prove useful in the event of subsequent SARS-CoV-2 mutations (Meneguzzo *et al.*, 2020). The *Citrus* oil market is expected to reach a valuation of 5.8 billion US dollars by 2032 (Future market insight report, 2021)

The *Citrus* genetic resources of India are enormous, both in cultivated and wild varieties. Although *Citrus* grows in practically every state in India, the Northeast is recognised as the "Treasure House of *Citrus* genetic wealth" (Caruso *et al.*, 2020). There is a tremendous pool of *Citrus* variation in wild and semi-wild varieties that are distributed without commercial cultivation or much care (Hazarika, 2012). The region is known for

producing a certain quality of *Citrus*, such as "Khasi Mandarin" or "Assam Sumthira", "Assam lemon", "Gol nemu" (a rough lemon), “Bira Jora” (a citron), many white and pink-flesh pummelos, etc (Bhattacharyya and Baruah, 1998). Khasi mandarin is the most popular *Citrus* cultivar in the country's north-eastern region because of its great quality, fruit colour, unique sugar-acid balance, and long storage life. Lemon is an important medicinal plant grown mostly for its alkaloids, that has anticancer properties. The antibacterial efficacy of crude extracts of different parts of Lemon (namely flower, stem, leaves and roots) against clinically relevant bacterial strains has been observed (Kawaii *et al.,* 2000). Assam lemon is one of the most important fruits of Assam. Kazi nemu (*Citrus limon*) is one of the main food items in every Assamese household and is exclusively grown and found only in Assam. Citron (*Citrus medica*) grows wild in the area and can be eaten raw or preserved as a pickle. It is locally known as Jora tenga and has different cultivars such as “Birajara,” “Banjara”, “Mithajara”, “Jaare Jamir”, and others. The largest *Citrus* fruit is the pummelo which is devoured by the people of this region for its appealing taste and its high fibre and nutritional content. Sour pummelos or *Citrus megaloxycarpa* is available in various forms such as “Bortenga”, “Hukmatenga”, “Holongtenga”, and “Jamir tenga”. *Citrus sinensis* (L.) Osbeck, sometimes known as sweet orange, is India's second most important cultivated species after mandarins. Its origins are assumed to be in southern China, north-eastern India, or perhaps southeastern Asia. Another traditional *Citrus* fruit found in Assam is grapefruit, which is used as a treatment for diabetes and low blood pressure (Barbora *et al*, 2020). The fruits of *Citrus latipes* (Khasi papeda) are rarely eaten despite being edible, and the fruits and leaves of *Citrus hystrix* (Kaffir lime) can be used in aromatherapy. The NE region's high *Citrus* genetic diversity is under imminent threat of extinction. Numerous wild and semi-wild *Citrus* species are under threat of extinction as a result of deforestation, as well as being often removed from farmers' fields in exchange for crops with higher economic value (Barbora *et al*, 2020).

Citrus and related genera are incredibly vital to the Indian fruit sector, hence the adequate recording of distinct species and cultivars, as well as the preservation of the endangered and threatened *Citrus* species is required. An estimation of their biochemical status is essential to find out and explore methods in which they may be used, not just for extracting health benefits, but also for industrial applications. There has not been any systematic biochemical study of the cultivars till date which is why it is imperative to estimate and document the compounds. The findings can encourage farmers to cultivate nutritionally superior cultivars and will help to popularize them for consumption. An interest

in the qualitative and quantitative content of the healthy compounds in *Citrus* fruit will also help to increase the nutraceutical potential of the crop. This in turn will result in increased investment in *Citrus* fruit farming and economic prospects.

Under the above considerations, a programmatic R&D effort has been undertaken on the different fruits of Citrus cultivars grown in Assam with the following objectives:

1. Biochemical analysis of some *Citrus* germplasm of Assam
2. Analysis of essential oils from *Citrus* peel.

Review of literature…

CHAPTER II

REVIEW OF LITERATURE

One of the most widely grown fruits in the world, *Citrus* is now a crop with significant worldwide economic importance. Their consumption has steadily increased with a growth in population. Our country holds an exclusive spot in the "*Citrus* belt of the world" due to her enormous wealth of *Citrus* genetic resources, both wild and farmed (Nair and Nayar, 1997). *Citrus* indigenous genetic resources are highly valuable in the *Citrus* industry. Recent investigations on *Citrus* genetic resources in north-eastern India revealed the presence of 23 species, one subspecies, and 68 varieties, giving this region a unique status as the "Treasure house of *Citrus* germplasm" (Sharma *et al.*, 2004). Because of widespread deforestation in the Himalayan area to provide the necessary ground for farming, wild populations of such species are dramatically declining (Ahuja, 1996). This demanded the exploration and collection of existing genetic diversity, as well as its conservation using proper methods.

Characterization is an integral part of every crop improvement programme. Even though numerous kinds of *Citrus* are cultivated in our country, the extensive genetic diversity of semi-wild and wild *Citrus* germplasm has only been sparingly utilised in crop enhancement programmes because of a lack of their characterization, which is primarily attributable to significant gaps in our understanding and knowledge of the useful traits of various *Citrus* species and varieties (Sharma *et al.*, 2004). *Citrus* is a very diversified fruit crop; therefore, extensive data evaluation and characterization are required to estimate the genetic diversity contained in these species. Especially, *Citrus* nutritional characterization studies are important in identifying its economic and therapeutic potential.

The work done in *Citrus* and other crops relevant to the proposed study is discussed below, under the following sub-headings:

2.1 Origin of *Citrus*

For a long time, the primitive centre of origin of the *Citrus* species has been a source of curiosity and debate. *Citrus* trees were first planted in "the tropical and subtropical regions of the Asian continent and the Malaysian archipelago" at least 4,000 years ago, although the exact origin of *Citrus* farming is still unknown (Berk, 2016). Many *Citrus* species, as well

as their relatives, are indigenous to south-eastern Asia, Australia, the Eastern Archipelago, New Guinea, New Caledonia, and Melanesia (Scora, 1975). In his pioneering study, Vavilov (1927) recognised two centres of the origin of *Citrus*, the Indo-Burma centre (Assam and Myanmar; citrons, mandarins and oranges) and the Indo-Malayan centre (Malay Archipelago and Indochinese Peninsula; pummelo). Tolkowsky (1938) refined this vision by emphasising the significance of the North-Eastern part of India and also aiming it directly towards the southern Chinese mountains. Tanaka (Swingle and Reece, 1967; Tanaka, 1959, 1961) in later investigations revised these concepts and concluded that northeast India and northern Myanmar was the primary centre, from where the crop dispersed to secondary centres in southeast China and Indo-China. For instance, Swingle and Reece (1967) proposed that a number of significant species of true *Citrus* first developed in a vast area of Southeast Asia that included South-eastern China, North-eastern India, and Myanmar. According to Wu *et al.* (2018), *Citrus* is typically regarded as having originated in Southeast Asia, particularly in Myanmar, the Yunnan region of South-west China, and the North-eastern Himalayan foothills of India.

2.2 Collection of *Citrus* germplasm

India is a centre of origin and diversity for *Citrus* and related genera. Tanaka (1928, 1937) and Bhattacharya and Dutta (1956) focused heavily on collecting *Citrus* genetic diversity from the northeastern region of our country throughout the third to fifth decade of the previous century. During this time, most of the new *Citrus* species were identified, gathered, and catalogued. Recent surveys and investigations documented the disappearance of several species from areas where they had previously been believed to exist (Singh and Singh, 2003). The genetic diversity of indigenous *Citrus* species is steadily eroding (Rai *et al.*, 1997). Therefore, the collection and conservation of the gene pool as genetic diversity is a prerequisite for crop development and improvement. In the Garo Hills of Meghalaya, there is an in-situ gene sanctuary for *Citrus*, which actively conserves the enormous diversity of *Citrus* in its native environment (Singh, 1981). The central and northwest Himalayas, the southern peninsula and Maharashtra are also diverse areas of *Citrus*. Ex-situ conservation of *Citrus* germplasm began in India in the 1950s. (Bhattacharya and Dutta, 1956) in field gene banks, but due to a lack of effective collection management and biotic stresses, the number of accessions kept has decreased. A new species *Citrus assamensis* was discovered by Bhattacharya and Dutta (1956) in Assam.

2.3 Nutritional and medicinal values

Citrus fruits are among the most nutritious fruits when it comes to their health benefits. *Citrus* fruits have several bioactivities, making therapeutic usage of them of great significance (Liu *et al.*, 2012). *Citrus* fruits are widely consumed across the world, in both fresh and processed forms. *Citrus* fruits are also a great source of minerals (selenium, zinc, copper, iron, and manganese), ascorbic acid (vitamin C), tocotrienols (vitamin E) and tocopherols, and various other nutrients (Cebadera *et al.*, 2019; Lu *et al.*, 2021; Zou *et al.*, 2016). Furthermore, the flavedo (outside layer) and albedo of *Citrus* fruits contain more flavonoids than the juice sacs (Multari *et al.*, 2020)

Citrus fruits are an abundant source of polyphenolic flavonoids, mainly flavanones, such as naringin, hesperidin, narirutin and neohesperidin (which account for almost 90% of all flavonoids) (Khan *et al.*, 2014). *Citrus* fruit flavonoids have been found to modulate lipid metabolism and adipocyte differentiation, minimise oxidative stress, enhance glucose tolerance as well as insulin sensitivity, inhibit inflammation and apoptosis, and improve endothelial dysfunction, all of which point to their potential antidiabetic effects (Kopustinskiene, 2020; Li *et al.*, 2017; Millar *et al.*, 2017; Mahmoud *et al.*, 2019; Rees *et al.*, 2018; Zaidun *et al.*, 2018; Zhang *et al.*, 2017). Hesperidin isolated from *C. sinensis* peels has a 36% antioxidant activity, according to research by Asjad *et al.* (2013). Foschi *et al.* (2010) showed that *Citrus* fruits have a protective role against cancers of the upper respiratory tract and digestive system. Consumption of *Citrus* fruits has been found to have an inverse dose relationship with the incidence of stomach cancer (Gonzalez *et al.*, 2012). A high diet of *Citrus* fruits has been shown to reduce the incidence of pancreatic cancer, stomach cancer and gastric cancer (Bae *et al.*, 2009). For individuals who fall into the highest consumption category, dietary intake of *Citrus* fruits offers a significant preventive function in the prevention of oral cancer (Cirmi *et al.*, 2018). It has been proposed that vitamin C helps prevent oxidative damage to DNA that results from inflammation, which is a factor in the development and spread of cancer. Additionally, vitamin C can destroy cancer cells because of its pro-oxidant properties (Grosso *et al.*, 2013; Block, 1991). *Citrus* flavonoids are powerful anti-hyperglycemic agents that work by binding to starch, boosting hepatic glycolysis and glycogen levels, and decreasing hepatic gluconeogenesis (Shen, 2012). Additionally, hesperidin, naringin, and nobiletin showed anti-diabetic effects, in part via reducing hepatic gluconeogenesis or enhancing insulin sensitivity in diabetic mice (Akiyama, 2010). According to a preliminary clinical trial, people with Alzheimer's disease may benefit from receiving decocted, nobiletin-rich *C. reticulata* peel orally for one year

without experiencing any negative side effects (Seki *et al.*, 2013). Another study (Sacco *et al.*, 2013) discovered that hesperidin provided a better balance in bone metabolism and bone health. In addition, *Citrus* fruits are a good source of polymethoxylated flavones, a special family of bioactive flavonoids (Saini *et al.*, 2022). *Citrus* fruit's immature peels have proven potential as a chemotherapeutic agent and are used to treat dyspepsia (Deyhim *et al.*, 2006; Kim *et al.*, 2008). The main flavanone in lemons and other *Citrus* species, hesperidin has analgesic and anti-inflammatory properties, affects vascular permeability, and modifies capillary resistance (Fuster, 1997). Numerous studies have proved the health benefits of *Citrus*-based foods for degenerative conditions, such as hypertension, cancer, diabetes, and cardiovascular disease (Gupta *et al.*, 2021; Park & Shin, 2021). *Citrus* fruits are an excellent supply of fibre, which helps with digestion. *Citrus* seed oil is used to create soap that showed exceptional anti-microbial, anti-fungal, anti-parasitic, and anti-oxidant capabilities (Atolani *et al.*, 2020). Following vitamin C intake, the skin's increased antioxidant state may be able to guard against oxidative stress brought on by environmental toxins and UV radiation (Valachhi *et al.*, 2015).

2.4 Morphological parameters

Morphological features are the oldest and most commonly used genetic markers, and they may be regarded as ideal for identifying specific germplasm. Morphological markers have the benefit of being simple, quick, and affordable tests and can be also performed on herbarium specimens and other dead tissues. Analysing morphology is a crucial part of classifying and evaluating *Citrus* diversity. Currently, physical traits are still taken into consideration and used as a first step in cultivar identification and diversity evaluation.

The fruit diameter of 30 pummelo accessions was found to range from 9.54 to18.94 cm (Rahman *et al.*, 2003) and that of bittersweet orange and sour orange was 73.7 mm and 60.4 mm respectively (Jaskani *et al.*, 2006). The fruit diameter of rangpur lime, rough lemon, and galgal ranged from 53.34 to 61.58 mm, 54.39 to 8.9 mm and 81 mm and 78 mm respectively (Singh and Singh, 2006). The fruit weight of pummelo accessions was 718 to 2160 g (Rahman *et al.*, 2003), of bitter-sweet orange was 195.9 g (Jaskani *et al.*, 2006), of 25 mandarin genotypes ranged from 59.46 to 266.33 g (Josan and Kaur, 2006) and that of 32 *Citrus* genotypes from Arunachal Pradesh ranged from 21.56 to 712.53 g (Rabha *et al.*, 2013). The number of segments for bitter-sweet orange and sour orange was 8.3 and 10.4 respectively (Jaskani *et al.*, 2006). Rind thickness for 30 pummelo accessions was 0.70 to 2.53 cm (Rahman *et al.*, 2005) and for rangpur lime was 2.17to 3.59 mm (Singh and Singh,

2006). The fruit shape of bittersweet orange and sour orange was ellipsoid (Jaskani *et al.*, 2006) and for rangpur lime it varied from ellipsoid, spheroid and oblique (Singh *et al.*, 2010). Rahman *et al.*, (2003) found that the epicarp colour of pummelo genotypes varied as greenish-yellow, yellow, reddish yellow and light yellow. The fruits of bitter orange and sour orange were found to have rough fruit textures (Jaskani *et al.*, 2006). In rangpur lime smooth and rough fruit textures were found (Singh and Singh., 2006). Solid, semi-solid and hollow fruit axis were found in rangpur lime cultivars studied by Singh *et al.* (2010) and sour orange was discovered to have a hollow fruit axis (Jaskani *et al.*, 2006). The seed shape in bitter-sweet orange varied from clavate to spheroid and it varied from clavate to cuneiform in sour orange (Jaskani *et al.*, 2006).

Among the accessions under his study, Fallahi *et al.* (1990) found that Foothill Lisbon had the greatest fruit weight, juice percentage, acid percentage, and percentages of fruit with smooth skin. Singh and Govind (1999) confirmed a varied dissimilarity in the physicochemical characteristics of 33 *Citrus* cultivars, with the segment number per fruit ranging from 8.5 to 17, the weight of the fruit ranging from 16.20 g to 1316.00 g and peel thickness ranging from 1.10 to 26.80 mm. Dubey (2000) examined the performance of 13 different varieties of sweet oranges and he found significant variations in the weight of fruit, thickness of peel, juice percentage, seed count, TSS, total acidity, and ascorbic acid concentration. Govind and Singh (2002) examined the quality of eight hybrids of *Citrus* and discovered that Troyer citrange had the thinnest (2.42 mm) peel, while Kara and Kinnow mandarin had the largest fruit weight (165.62 g) and the number of segments per fruit (12.07). Sweet orange (*Citrus sinensis*) varieties exhibit significant variation in fruit weight (145g/ fruit to 226 g/ fruit), TSS (7.00% to 9.90%), juice content (45.62% to 50.41%), ascorbic acid (18.22 mg/100 ml to 26.40 mg/100 ml), acid content (0.42% to 1.03%) and seeds per fruit (0 to11 seeds per fruit), according to research by Josan and Kaur (2004). Khan *et al.* (2005) examined the physicochemical characteristics of 20 sweet orange varieties and discovered that Mosambi had the maximum percent juice content (68.80%) while Joppa had the highest fruit weight (247.00 g), fruit length (8.10 cm), fruit diameter (7.90 cm), and number of seeds (28.00).

2.5 Moisture content (%)

Moisture content is an important factor in food materials, as it refers to the amount of water that can be found in a given sample. The moisture of a substance is comprised of all the components that evaporate when heated and cause weight loss in the sample. Water

is an essential element in all parts of the body, as it plays a major role in various bodily functions. Consequently, it is important to make sure that the moisture content in food materials is adequate to meet the needs of the body. It carries nutrients, transports waste products from cells in the body, helps in the digestion and absorption of food, and regulates body temperature (Johnson, 1996). When the moisture content of *Citrus* plants is too low, it can cause the trees to become stressed and less productive. The fruits may dry out, and the flavour of the fruit can be affected. The water content in the fresh pulp of oranges and lemons was documented to be 85.7% and 88.6% respectively (Tripodo *et al*., 2004). According to Haque *et al.* (2009), the moisture content of pummelo (*C. maxima*) is around 90.23 per cent. M'hiri *et al.* (2015) reported that moisture content in *Citrus* peel is 75.3 ± 10.2 % and that in *Citrus* pulp is 85.7 ± 0.00 %. Gaikwad *et al.* (2015) assessed the moisture content of 30 pummelo genotypes, which ranged from 82.1 to 91.05 per cent. An extremely low moisture content of 23.75% was reported in rough lemon (Mohammed *et al.*, 2013). In *Citrus natsudaidai* peels, a high level of moisture content of 80% was documented by Matsuo *et al.* (2019).

2.6 pH

The pH of *Citrus* fruits vary depending on the type of *Citrus* fruit. The pH of *Citrus sinensis* fruit juice was reported to be 3.8% (Shravan, 2018). Gaikwad (2018) carried out the morphological characterization of 30 *Citrus* rootstock genotypes from Maharashtra and discovered that the pH of the fruit juice varied from 2.09 to 4.48. Sayed *et al.* (2006) carried out a morphological and physiochemical characterization of 10 lime and lemon accessions and it was discovered that the pH varied from 6.20-2.12 with the highest in Succari lime and lowest in ponderosa cultivar. In another study conducted by Mansour *et al.* (2019), the pH of *Citrus aurantium* juice was lower in unripe fruits than in mature fruits, indicating that unripe fruits were more acidic than ripe fruits. Raghavan *et al.* (2022) reported that the pH in mandarin varieties from North-east India varied from 2.27 to 4.74. In the 13 Mandarin types examined by Hassan *et al.* (2008), the pH was found to vary from 3.12 to 3.91. Sgroppo *et al.* (2015) measured the pH of grapefruits from Argentina and Paraguay and found that the range was 3.41-4.16. Similarly, Debbabi *et al.* (2013) discovered pH variance in 28 sweet orange cultivars ranging from 2.64-5.95. Herath *et al.* (2016) found that the pH of fruits from eleven *Citrus* genotypes ranged from 2.37 to 3.98.

2.7 Total Soluble Solids (°Brix)

Total soluble solids (TSS) are an important indicator of the quality of *Citrus* fruits. TSS is a measure of the soluble substances that are present in the juice of the fruit. Total soluble solids (TSS) and acid content, as well as the delicate balance between them, have a significant impact on the flavour and interior quality of *Citrus* fruits (Ladaniya, 2008). The TSS of several pummelo genotypes ranged from 7.08 to 10°Brix (Singh, 2004). This was in accordance with the findings by Patil and Reddy (2008) who found that the TSS varied from 7.8 to 10.13° Brix across 12 pummelo clones. Furthermore, the TSS of 30 pummelo accessions from Bangladesh was studied which ranged from 6.16 to 9.66°Brix (Rahman *et al.*, 2003). The TSS across 18 types of sweet orange from Punjab ranged from 7.00 to 9.90° Brix (Josan and Kaur, 2004). Similarly, the total soluble solid content of 25 Mandarin cultivars from Punjab ranged from 6.13 to 12.20 Brix (Josan and Kaur, 2006). The total soluble solids in mandarin fruits from six districts in Bhutan varied from 10.4 to 12.9°Brix as reported by Dorji and Yapwattanaphun (2011). The Total soluble solids varied from 6.3-10.5°Brix in Rough lemon, 7.29-8.91°Brix in rangpur lime, 9.5°Brix in Galgal and 7.1°Brix in Alemow, respectively, (Singh and Singh, 2006). The total soluble solid content of 28 sweet orange cultivars investigated by Debbabi *et al.* (2013) ranged from 9.20 to 15.4°Brix. Akhter *et al.* (2009) conducted an experiment on 14 Jamir accessions (*Citrus jambhiri*) and documented the proportion of total soluble solids (TSS) which varied from 11.03% (CJ04) to 12.20% (CJ12) with an average value of 11.34%. According to the experiment conducted by Marboh *et al.* (2015), TSS ranged from 5.88 to 6.80 per cent across different cultivars of rough lemon, galgal and rangpur lime. The TSS in orange, sweet lime and lemon was found to be 11.47, 4.5 and 4.5° Brix, respectively (Jamil *et al.*, 2015). *Citrus* genotypes exhibit substantial diversity in the ratio of TSS to acid, as observed by Khan *et al.* (2010) in sweet orange and Verma *et al.* (2012) in Mandarin.

2.8 Titratable Acidity (%)

Titratable acidity in fruit juices is determined by neutralizing the fruit juice with a strong base solution to a fixed pH to assess the amount of titratable hydrogen ions contained in the samples (Tyl and Sadler, 2017). All the acidic components of fruit juice, including free hydrogen ions, acid salts, cations and organic acids are included in this value**.** In addition, the ripeness of the fruit also affects the titratable acidity. As the fruit ripens, the amount of acid decreases (Zekri *et al.*, 2011). Titratable acidity is an important characteristic in judging the quality of *Citrus* fruits (Ladaniya, 2008).

The acidity of *Citrus jambhiri* fruits ranges from 1.17% to 7.19% (Sharma *et al.*, (2004). Similarly, Josan and Kaur (2006) determined the acidity of 25 mandarin cultivars, which ranged from 0.41 to 1.43 per cent. Gaikwad *et al* (2018) examined 30 *Citrus* rootstock genotypes representing four species in Maharashtra, and noted that the total acidity ranged from 2.44 to 8.72%. Kakoti *et al* (2019) conducted a study on the status of Khasi mandarin in Meghalaya and reported that the average acidity was 0.65%. Furthermore, they also investigated the biochemical characteristics of Khasi mandarin in the state of Tripura (2019) and found similar values of average acidity. Hangsing *et al.* (2016) conducted a study on the performance of Khasi mandarin in the Garo hills of Meghalaya by grouping the trees into four groups based on the age of the trees from 10 to 40 years and reported that acidity ranged from 0.73 to 0.81 per cent. The rough lemon acidity ranged from 4.28 to 5.16 per cent, rangpur lime acidity ranged from 4.87 to 5.50 per cent, and for Galgal, the acidity ranged from 5.13 to 3.84 per cent (Marboh *et al.*, 2015). The acidity of *Citrus* fruits as determined by Singh and Singh (2006) was 3.36-5.45 per cent in rough lemon fruits, 3.9-5.28 per cent in rangpur lime, 4.1 per cent in Galgal and 4.2 per cent in Alemow. According to Singh *et al.* (2010), the acidity ranged from 3.4 to 5.0 percent across six rangpur lime genotypes. Furthermore, the acidity ranged from 0.11 to 1.45 percent in 32 *Citrus* genotypes from Arunachal Pradesh (Rabha *et al.*, 2013). Paudyal and Haq (2008) found low acidity (0.81-1.02%) in six pummelo varieties. According to Singh *et al.* (2009), the average acidity of 28 hill lemon genotypes was around 6.06 per cent.

2.9 Ash Content (%)

The amount of mineral matter left behind when a sample is incinerated at a high temperature is referred to as ash content. It measures the mineral content of food and is represented as a percentage of dry weight. A fruit's ash level varies based on its kind, variety, and maturity. The ash content in *Citrus* sinensis collected from Dutsinma, Katsina State, Nigeria was 14.76 ± 0.001% as reported by Joseph, 2016. The ash content in orange and sweet lime peels was 6.50% and 7.82%, respectively (Indulekha *et al.*, 2017)**.** The crude ash content of dried sweet lime peels (*Citrus limetta*) was found to be 5.93% (Suri *et al.*, 2022). Mathias *et al.* (2019) discovered up to 5.57% ash level in dried *Citrus* peels from Goa in another investigation.

2.10 Crude Protein (%)

Citrus fruits are a great source of many essential nutrients, including protein. Crude protein is the total protein content of food material determined as a factor of the total nitrogen

content of food proteins. Protein is necessary for the maintenance and repair of muscle tissue, and helps feeling full for longer. Those attempting to maintain a healthy lifestyle or reduce weight may find this to be very helpful.

The average amount of protein in orange and lemon pulp that grew in the province of Messina in Italy was 8.6% and 7.6% respectively (Tripodo, 2004). Among eight varieties of *Citrus* pulp from Spain, the crude protein ranged from 5.0-8.0 % with the lowest in navel and the highest in mandarina (Pascual *et al.,* 1980). 3.1% crude protein was present in flour samples extracted from dried sweet orange (*Citrus sinensis)* seeds (Akpata and Akubor, 1999). Sweet orange rinds which are a major waste product of *Citrus* are rich in protein content (7.15%) (Olabinjo *et al.,* 2017). Osarumwense *et al.* (2013) stated that the protein content in *Citrus* sinensis peels ranged from 4.05 ± 0.25%. According to another study done by M'hiri *et al* (2015), the protein content of Tunisian Maltese orange peel was 8.120 ± 0.120 g/100g. In *Citrus sinensis* (sweet orange), the crude protein in seed, flavedo and albedo were 6.13 ± 0.51%, 3.94 ± 0.25 %, and 0.88 ± 0.05 % respectively. In peels of sweet lime, the crude protein percentage was 8.18% (Suri *et al.,* 2022). They additionally stated that the low crude protein content can be attributed to the mallard reaction or tannin synthesis with protein, which reduces protein availability and lowers protein concentration in peels.

2.11 Sugar (%)

Sugars play a major role in citrus fruit physiology when the fruit is attached to the tree and also after its harvest (Ladaniya, 2008). Sugars are crucial for the growth and development of plants. They not only serve as an energy source but also act as hormones in signal transduction (Li and Sheen, 2016). They also determine the flavour and quality of the fruits (Liu *et al.,* 2022). The reducing and non-reducing sugars are both important components of a healthy diet and are abundant in fruits. Reducing sugars are the simplest forms of sugars and they have a free aldehyde or ketonic group in them whereas non-reducing sugars do not possess free aldehyde or ketonic groups (Chesworth, 1998). Reducing sugars are the most easily digested and absorbed among all types of sugars. This is because they do not need to be broken down into individual molecules before they can be absorbed (BeMiller, 2017). As a result, they provide a quick source of energy, making them ideal for those who need a burst of energy. However, in order to be absorbed, non-reducing sugars need to be broken down into individual molecules. This process takes more time and energy but can result in a sustained release of energy over a longer period. As a result of the fibre

that they contain, whole fruit sugars are typically not linked to weight gain (Kumar *et al.,* 2020).

The reducing and non-reducing sugar in fruits of different pummelo genotypes from Maharashtra ranged from 1.47-2.9% and 1.75-4.48% respectively (Gaikwad *et al.,* 2015). This was in accordance with the sugar content found in 35 genotypes of Pummelo from Assam which varied from 3.23- 5.59% for reducing sugars and 2.70-5.95% for non-reducing sugars respectively (Hazarika *et al.,* 2012). Among 30 *Citrus* rootstock genotypes of *Citrus* representing 4 species (*C. jambhiri, C. limonia, C. pseudolimon and C. macrophylla*), reducing and non-reducing sugar content varied from 1.35 to 2.90 % and 0.88 to 3.93 % respectively (Gaikwad *et al.,* 2018). Among 32 *Citrus* genotypes of Arunachal Pradesh, the reducing sugar ranged from 0.05-4% with a mean of 1.20 % (Rabha *et al.,* 2013). The reducing sugars in mandarin genotypes ranged from 4.41-6.52 per cent as reported by Kumar *et al.* (2011). Similarly, Shravan *et al.* (2018) documented that the reducing and non-reducing sugar content in sweet orange (*Citrus sinensis*) fruits were 1.8% and 6.56% respectively. As per Diwan *et al.* (2014), reducing sugar in 28 sweet orange varieties varied from 3.29 to 4.70%, with a mean of 4.08. The non-reducing sugar fluctuated from 1.82-3.32% with an average of 2.62%. The reducing sugar percentage of Khasi mandarin grown in the Garo hills of Meghalaya was greatest in trees of 10-20 years of age (7.84%), whereas the non-reducing sugar percentage was highest in trees of 21-30 years of age (2.76%) (Hangsing *et al*, 2016). Deshmukh *et al.* (2016) reported an increasing trend in the sugar content of Khasi mandarin at different altitudes. The non-reducing sugar increased from 3.14 to 3.96%, 2.83 to 3.88%, 2.54 to 4.00% and 2.88 to 4.03 % and total sugar increased from 4.80 to 6.01%, 4.52 to 6.03%, 4.15 to 6.12% and 4.65 to 6.11% at 500 - 600m, 700-800m, 900-1000m and 1300 to 1400m altitude respectively.

2.12 Organic acids (mg/ 100 ml)

For food and beverage technology and quality assessment, it is deemed crucial to identify and quantitatively analyse the primary organic acids in fruits (Hasib *et al.,* 2002). *Citrus* fruits are well known for their high levels of citric acid, but they contain other organic acids as well. These acids include malic acid, tartaric acid, and ascorbic acid. In an experiment by Albertni *et al.* (2006) regardless of the variety, quinic acid was the dominant organic acid for the first 50 days of growth. However, after that, citric acid took over in acidic varieties while malic acid surpassed it in acid-less types. Ascorbic acid is the main antioxidant in Citrus, according to a recent study that examined fifteen distinct Citrus

cultivars (Xu *et al.*, 2008). The ascorbic acid content of lime, oranges, and grapefruit was found to be around 47.9 mg/100 g, 47.7 mg/100 g and 35.1 mg/100 g, respectively (Gorinstein *et al.*, 2001). An experiment was conducted by Shrestha *et al.* (2016) on the different *Citrus* fruits of Kathmandu Valley and it was reported that the average ascorbic acid content was found to be highest in pummelo at approximately 61.29 mg/100 ml and lowest in citron at around 17.4 mg/100 ml. The ascorbic acid concentration in the juice of 13 mandarin types ranged from 14.0 to 44.8 mg per 100 ml (Hassan *et al.*, 2008)

Oxalic, malic, malonic and citric acid was found to be predominant in the peel of the *Citrus* fruits and malic acid, citric acid, tartaric acid, quinic acid, succinic acid, oxalic acid, and ascorbic acid were the seven organic acids that were shown to contribute to the acidity of *Citrus* fruit pulp (Albertini *et al.*, 2006). Violeta *et al.* (2010) conducted an experiment on eight species of *Citrus* fruit juices by reverse phase HPLC and found out that citric acid percentage was maximum and it ranged from 6.88 to 73.93 g l^{-1}. Citric acid, malic acid, lactic acid and ascorbic acid were highest in lemon, lime, sweetie grapefruit and lemon respectively. Kardeniz (2004) investigated the distribution of organic acids in 19 authentic Turkish orange juice samples. Citric acid, with concentrations ranging from 6.05 to 60.32 g/l in all *Citrus* juices except for Clementine tangerine type, was the most prevalent organic acid. Fumaric acid (0-807 g l^{-1}) and malic acid (1.27-12.15 g l^{-1}) were the next two most prevalent acids. By using silica gel column chromatography, the organic acids in the juice of 47 samples of 42 *Citrus* species were identified and citrate was found as the most abundant organic acid among all the *Citrus* species studied, accounting for 75.4 to 96.9% of total recoverable acids (Yamaki, 1989). Ascorbic acid (137-251 g mL^{-1}) and citric acid (5-22 mg mL^{-1}) were discovered to be present in large amounts in pummelo as well as Rio Red grapefruit (Uckoo, 2011). It was discovered that organically grown *Citrus* contains more organic acids than conventionally grown fruit (citric, malic, tartaric, ascorbic, and malonic) but fruits from conventional cultivation had a greater oxalic acid content (Duarte *et al.*, 2010). Five organic acids, including oxalic, citric, ascorbic, malic, and succinic acids, were found in grapefruit juice and the amount of citric acid, which was the main organic acid present in grapefruit juice, ranged from 18.75 g L^{-1} in Rio red to 23.89 g L^{-1} in Star ruby (Kelebek, 2010). This was in accordance with earlier published findings about the levels of citric acid in Handerson, Marsh seedless, and Star ruby (which were, respectively, 19.36, 24.25, and 16.96 g L^{-1}) (Kardeniz, 2004).

2.13 Water-soluble Vitamins (mg/ 100 ml)

Citrus fruits are well-known for their high vitamin content. *Citrus* fruits contain a variety of vitamins such as vitamin A, vitamin B_6, vitamin C, thiamine, folate, and pantothenic acid (Liu *et al.*, 2012). Vitamin A is present in appreciable quantity as the carotenoid provitamin A in *Citrus*. The riboflavin content in the pineapple variety and valencia variety of orange was 16 µg/100 ml and 15 µg/100 ml juice and similarly, the riboflavin content in seeded and seedless grapefruit was 12 µg/100 ml and 11 µg/ 100 ml juice (Bailey, 1942). The Thiamine content in oranges ranged from 54-81 µg/100 ml juice and that in grapefruit ranged from 18-47 µg/100 ml (Bailey, 1942)

Braddock (1972) reported only 0.1 µg of Vitamin E in 100 ml of orange juice. Furthermore, in Dancy tangerine and Valencia oranges, cryptoxanthin is the main vitamin A precursor (Curl *et al.*, 1954). Nearly 5/6 of all ascorbic acid in a grapefruit and 3/4 in an orange is found in the peel (Atkins *et al.*, 1945). Ani and Abel (2018) found that in fruit juice and peel extract of *Citrus maxima*, the thiamine content was 0.55 ± 0.20 mg/100 g in juice and 11.20 ± 2.34 mg/100 g in the peel, niacin content was 14.42 ± 1.71 mg/100 ml in juice and 224.16 ± 22.99 mg/100 g in the peel, vitamin C content was 26.36 ± 3.19 mg/100g in juice and 19.34 ± 3.75 mg/100 g in the peel, and vitamin E content was 2.11 ± 0.08 mg/100 g in juice and 4.45 ± 0.07b mg/100 g in the peel.

2.14 Mineral constituents (mg/100 g)

Citrus fruit pulp and peel are both good sources of macro- and micronutrients. Orange, lime, and mandarin peels as well as their pulps are potential sources of minerals that may be employed for their health benefits in food items and can be used as a source of functional compounds (Barros *et al.,* 2012). *Citrus sinensis* peel revealed the existence of some trace elements in high concentrations, including Zn (14.04 + 0.96) and Mg (15.55 + 1.45) and several heavy metals in lower amounts such as Cd (0.001 + 0.00), Pd (0.08 + 0.005), and Ni (0.05 + 0.00) mg/L (Osarumwense *et al.* 2013). The mineral composition of *Citrus maxima* fruit was revealed in the order Ca > Na > Ph > Fe > Mg > K in the juice and Ca > Ph > Na > Fe > K > Mg in the peel extract (Ani and Abel, 2018). Mansour (2019) reported that the mineral content of *Citrus aurantium* fruits decreased with maturity except for Na. According to him, in *Citrus auratium*, the green flavedo has the highest concentration of minerals such as K, Mg, Fe, Cu, and Mn, while juice has the lowest and Ca and Zn were highest in green albedo. It has been reported that lime and orange pulps have high potassium levels (152 mg/ 100 g and 154/ 100 g respectively), sodium and calcium content is found

high in lime pulp (3.88 mg/ 100 g and 63.9 mg/ 100 g respectively), phosphorous content is high in orange pulp (25.3 mg/ 100 g), iron and magnesium is high in pomelo pulp (0.52 mg/ 100 g and 23 mg/ 100g respectively) and pomelo and oranges were found to be very high in copper (0.21 mg/ 100 g) and manganese (0.15 mg/ 100 g) (Czech, 2020). The concentrations of Fe, Zn, Cu, and Mn in fresh fruits of Jaffa Sweeties, a new variety of *Citrus*, were 1079–1331 g/kg, 661–822 g/kg, 348–532 g/kg, and 99–171 g/ kg respectively (Gorinstein *et al.*, 2004).

2.15 Essential oil content (%)

In the past, *Citrus* pulps were utilized for culinary (healthy food) purposes, whilst *Citrus* peels as a byproduct were only of limited value, such as a source of fibre or animal feed (Chau & Huang, 2003). Fruit peels generally guard the seeds and pulp needed for reproduction, and different phytochemicals allow fruits to thrive and defend themselves from outside dangers (Molyneux *et al.*, 2007). *Citrus* essential oils, which are mostly concentrated in the oil glands of *Citrus* peels, are a significant biologically active material (Hou *et al.*, 2019). According to Singh *et al.* (2010), monoterpenic essential oils are natural antioxidants. They make up, on average, 0.5–5% of the fresh weight of *Citrus* peels (Liu *et al.*, 2021). Essential oils are the most significant *Citrus* by-products derived from peels. They are often utilized as flavouring ingredients in beverages, ice cream, pastries, air fresheners, home goods, and cosmetics (Ferhat *et al.*, 2006). The oil content of *Citrus* fruits varied depending on the variety and species. The *Citrus* oil was also noted to vary with the size and maturity of the fruit. The release of volatile compounds increases with rising temperature, maturity and ruptured peel and juice components (Ladaniya, 2008). *Citrus* paradisi (*Citrus sinesnis* var. malta) had the highest percentage of essential oil extraction (0.45%), followed by *Citrus reticulate* var. Mandarin (0.33%) and Tangerine (*Citrus mousami*) (0.37%), according to a study on the extraction of essential oils from five different *Citrus* varieties viz. Mousami, Mandarin, Tangerine, Grapefruit and Malta and all five *Citrus* oils were found to contain between 50-80 per cent of alkaloids, tannins, sterols, terpenoids, saponins, and flavonoids during phytochemical analysis (Javed *et al.*, 2014). Hydro distillation was utilised for the extraction of essential oils from the leaves of orange, lemon, mandarin and brigadier and the yields obtained were 0.96, 1.02, 0.51 and 0.73% respectively (Zohra *et al.*, 2015)

A yellow-coloured essential oil was extracted from the peels of Lemon (*Citrus limon*) whose yield was 1.33% and DL-limonene was the main component of the essential oil with a percentage of 46.93% (Moosavy *et al.*, 2017). In grapefruit, (*Citrus* × *paradisi* var. red

blush), the highest percentage of essential oil (0.43% v/w) was found at the yellowish-green stage and the lowest amount (0.14% v/w) was found at the green stage (Ghani *et al.*, 2021). The essential oil percentage in *Citrus pyriformis* Hassk (Ponderosa lemon) and *Citrus jambhiri* Lush. (Rough lemon) were found to be 4% and 1.2% (v/w), respectively and a total of 94 compounds were detected from the essential oils (Hamdan *et al.*, 2010). The hydro-distilled essential oil content from peels of *C. reticulata, C. sinensis* and *C. paradisii* were 0.30, 0.24 and 0.20 g/100g, respectively (Kamal *et al.*, 2013) The findings of the current study are consistent with the findings of Tu *et al.* (2002), who discovered that the production of *Citrus* essential oils varied with different plant species, ranging in most cases from 0.2-2.0%.

2.16 Volatile compounds in essential oil (Relative %)

Citrus essential oils are a mixture of complex hydrocarbons, oxygenated derivatives of terpenoid and non-terpenoid origin, functional groups like aldehydes, alcohols, and ketones, as well as other intricate compounds like esters and organic acids (Merle *et al.*, 2004). Essential oils from *Citrus* contain a wide variety of compounds, ranging from 20 to 60 numbers per oil (Bakkali *et al*., 2008). Volatile chemicals make up 85%–99% of these, whereas nonvolatile compounds make up the remaining 1%–15%. D-Limonene, a hydrocarbon monoterpene, is consistently the most abundant molecule in the essential oils extracted from *Citrus* rinds, making up typically between 60 and 95 per cent of the oil (Jing *et al*., 2014). The essential oil of *Citrus karna* was examined by Malhotra *et al*. (2008), who found that it included D-limonene (92.31%), the principal chemical ingredient, as well as other minor compounds such α-pinene (1.23%) and β-pinene (1.80%). Kamal *et al*., (2013) discovered that β-myrcene and limonene were the predominant components in the peels of three *Citrus* species: *C. reticulate, C. sinensis,* and *C. paradisii*. The oil's flavonoid component possesses antioxidant, antimicrobial, antibacterial, and anti-inflammatory properties, as well as the ability to block particular enzymes, stimulate neurotransmitters and hormones, and scavenge free radicals (Ezeabara *et al.*, 2013). *Citrus* essential oils are also used as insect-repellent, larvicidal, antiviral, antihepatotoxic, and antimutagenic agent (Kanaze *et al*., 2009). Instead of a synthetic and inorganic chemical-derived insecticide, bioactive compounds of *Citrus*, especially essential oils can be used as a substitute (Abad *et al*., 2014) The essential alcohols in orange oil that contribute to its flavour are linalool, a-terpineol, and terpinene-4-ol (Shaw, 1979). Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil from Kaffir lime (*C. hystrix*) revealed that pinene, sabinene, limonene, and citronellal were the principal ingredients, with concentrations of 24.62%,

22.06%, 19.29%, and 10.58%, respectively (Suwannayod *et al*., 2018). Mehmood *et al*. (2019) discovered that the antimicrobial activity of peel essential oil of ripened and unripened *C. limon* had an inhibitory effect against four human pathogenic bacteria viz., *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium,* and *Staphylococcus aureus*. The antibacterial activity of *Citrus maxima* essential oil against human pathogenic bacteria (*E. coli, B. subtilis, S. aureus, P. aeruginosa, B. licheniformis*, and *B. altitudinosa*) was examined and it was found that the essential oil inhibited all the strains. (Chen *et al*., 2010) In order to reduce fungus growth and contamination and increase the shelf life of different foods, *Citrus* essential oils can be used as a natural antifungal agent. The potential uses of *Citrus* essential oils in packaging, preservation, and food safety are enormous. Gelatin films with *Citrus* essential oils added have improved physicochemical qualities as well as antibacterial action. (Bora *et al*., 2020)

Materials and Methods… ✍

CHAPTER III

MATERIALS AND METHODS

The information related to the materials utilized and the procedures followed as per the experimental details during the present investigation are presented in this chapter.

3.1 COLLECTION OF PLANT SAMPLE

Fully developed citrus fruits were collected from AAU-Citrus and Plantation Crops Research Station, Tinsukia and CCRI (ICAR), BNCA Campus, Biswanath Chariali Assam. Following harvesting, the undamaged fruits were cleaned and processed for various laboratory experiments.

A total of ten species of *Citrus* representing all five important groups of *Citrus* were collected.

TABLE 3. 1: *CITRUS* GENOTYPES USED FOR THE PRESENT STUDY

Sl. no	***Group name***	***Crop name***	***Botanical name***	***Vernacular name***
1	Acid group	Rangpur lime	*Citrus limonia*	Rangpur lime
		Citron	*Citrus medica*	Bira-jora
2	Orange group	Sweet orange	*Citrus sinensis*	Mithachakola
		Sour orange	*Citrus aurantium*	Karun jamir
3	Pummelo and grapefruit group	Grapefruit	*Citrus paradisi*	Soh- khayllah
		Pummelo	*Citrus grandis*	Pummelo red
4	Mandarin group	Khasi mandarin	*Citrus reticulata*	Komola tenga
		Cleopatra Mandarin	*Citrus reshni*	Cleopatra mandarin
5	Other species	Khasi papeda	*Citrus latipes*	Khasi papeda
		Kaffir lime	*Citrus hystrix*	Kaffir lime

3.2 PLACE OF WORK

The present study was carried out in the Laboratories of Department of Horticulture and Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat.

3.3 PROCESSING OF PLANT SAMPLE

The fruits were evaluated for their morphological parameters. The juice was extracted by delicately squeezing the fruits after cutting them in half and filtering using a muslin cloth before being collected in clean containers. The seeds were removed and collected in clean bottles. The peel was removed and placed in zip bags and stored at -80˚C for future analysis.

3.4 MORPHOLOGICAL CHARACTERISTICS

3.4.1 Fruit weight (g)

Three mature fruits were chosen at random from each species, and then the mean weight in grams was determined using an analytical balance.

3.4.2 Fruit length (cm)

Using a pair of vernier callipers, the lengths of three randomly chosen mature fruits from every species were measured and recorded.

3.4.3 Fruit breadth (cm)

Vernier callipers were used to measure the diameter of three randomly chosen mature fruits from every species and the results were recorded.

3.4.4 Number of segments

The number of segments for three ripe fruits from each species was counted and recorded.

3.4.5 Fruit rind thickness (mm)

The rind thickness of fruits belonging to each species was measured with a pair of vernier callipers and recorded in mm.

3.4.6 Juice content (%)

Juice was extracted from the fruits and juice content (%) was calculated as per the following formula:

$$\text{Juice content (\%)} = \frac{\text{Net juice weight} \times 100}{\text{Fruit weight}}$$

3.4.7 Fruit shape

Fruit shape was documented for each species using the Descriptors for Citrus (IPGRI, 1999) and was rated as spheroid, ellipsoid, pyriform, oblique (asymmetric), obloid and ovoid.

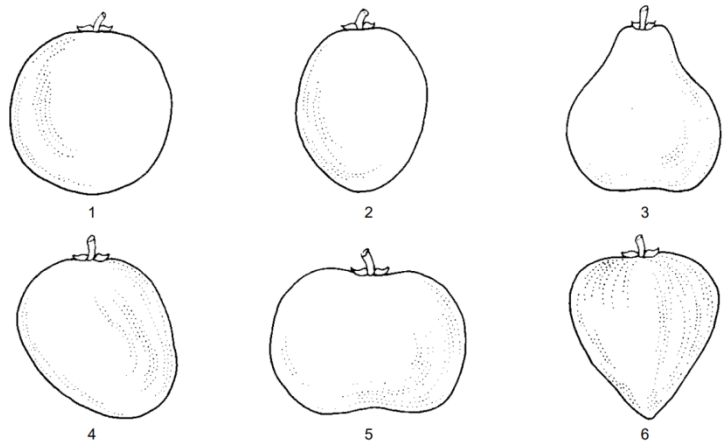


FIG 3. 1: FRUIT SHAPE

(1: spheroid; 2: ellipsoid; 3: pyriform; 4: oblique (asymmetric); 5: obolid; 6: ovoid)

3.4.8 Shape of fruit base

The fruit base shape was examined in five ripe fruits from each species at random and categorised as necked, convex, concave, truncate, concave collared, and collared with neck (Descriptors for Citrus,1999).

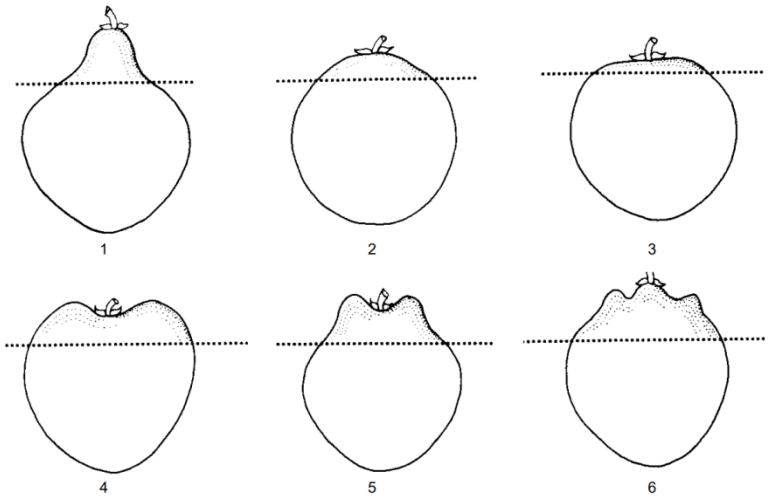


FIG 3. 2: SHAPE OF FRUIT BASE

(1: necked; 2: convex; 3: concave; 4: truncate;
5: concave collared; 6: collared with neck)

3.4.9 Shape of fruit apex

The fruit apex of five ripe fruits from each species was observed and categorised as mammiform, acute, rounded, truncate, or depressed (Descriptors for Citrus,1999).

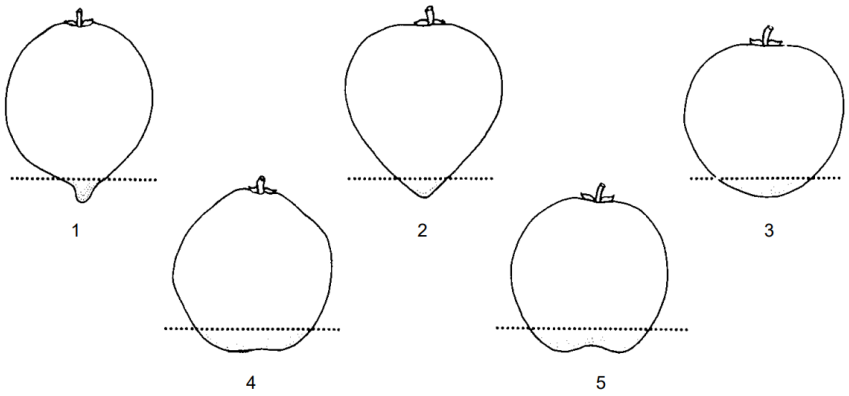


FIG 3. 3: SHAPE OF FRUIT APEX

(1: mammiform; 2: acute; 3: rounded; 4:
truncate; 5: depressed)

3.4.10 Fruit skin (epicarp colour)

The colour of the fruit was visually observed and recorded as Green, Green-yellow, Light- yellow, Dark-yellow, Light-orange, Orange, Dark-orange, pink-yellow, orange, pink-orange, red, red-orange, or other (Descriptors for Citrus,1999).

3.4.11 Fruit surface texture

Five fruits were chosen at random from each species to assess their surface texture, which was graded as rough, smooth, grooved, papillate, bumpy or pitted (Descriptors for Citrus,1999).

3.4.12 Albedo colour

The albedo colour of each species was visually observed and rated as red, orange, pink, yellow, white, or green (Descriptors for Citrus,1999).

3.4.13 Adherence of albedo

The adherence of the mesocarp to the endocarp was visually observed and recorded as weak, medium or strong (Descriptors for Citrus,1999).

3.4.14 Fruit axis

The axis of the fruit was recorded as hollow, semi-hollow or solid for every species (Descriptors for Citrus,1999).

3.4.15 Pulp colour

The colour of the pulp was visually observed for each species and recorded as per the Descriptor of Citrus, 1999.

3.4.16 Pulp colour intensity

The intensity of pulp colour was perceived in five fruits for each species and recorded as light or dark (Descriptors for Citrus,1999).

3.4.17 Seeds per fruit

The seeds were manually counted and noted after being removed from the fruits.

3.4.18 Seed shape

The shape of the seed was visually assessed after extracting the seeds from each species and classified as fusiform, clavate, cuneiform, ovoid, semi-deltoid, spheroid, and semi-spheroid (Descriptors for Citrus,1999).

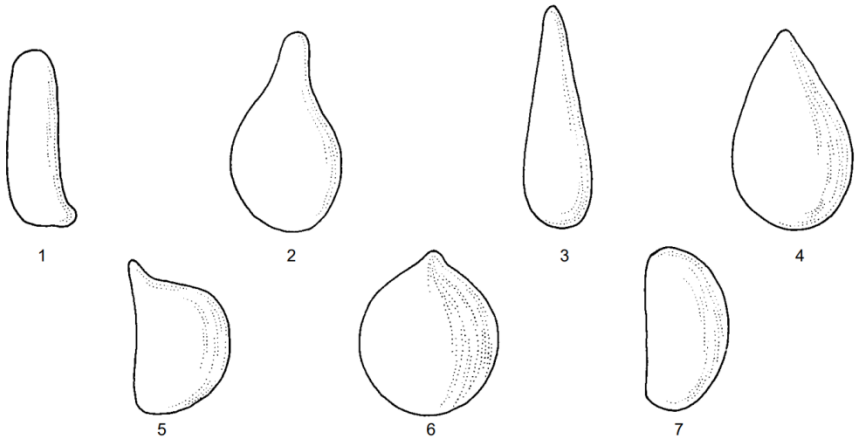


FIG 3.4: SEED SHAPE

(1: fusiform; 2: clavate; 3: cuneiform; 4: ovoid; 5: semi-deltoid; 6: spheroid; 7: semi-spheroid)

3.4.18 Seed colour

The colour of the seed was observed visually for seeds of each species and classified as brown, green, yellowish, cream and white (Descriptors for Citrus,1999).

3.5 BIOCHEMICAL CHARACTERISTICS

3.5.1 Moisture content (%)

With the use of an electronic moisture balance (Shimadzu MOC-120H), the moisture content of the peel and pulp was calculated and expressed as a percentage.

3.5.2 Juice pH

The pH of fruit juice after extraction was quantified using a pH meter.

3.5.3 Total soluble solids (TSS) (°Brix)

The total soluble solids (TSS) of the fruit juice were estimated with the aid of a digital refractometer (Milwaukee Digital Brix Refractometer - MA871). A drop of fruit juice was placed in the prism carefully and the reading that was expressed in °Brix was noted.

3.5.4 Titratable acidity (%)

Titratable acidity was measured by adopting the standard method of the Association of Analytical Chemists (A.O.A.C., 1980). 10 ml of the clear juice was taken and it was diluted with distilled water up to 100 ml and filtered. 10 ml of the filtered solution was titrated against the standard 0.1 N NaOH solution. The indicator used was phenolphthalein. The appearance of a light pink shade was considered the endpoint of the titration. Titratable acidity was expressed as a percentage of citric acid and was calculated with the help of the following formula.

$$\text{Titratable acidity (\%)} = \frac{\text{Normality of NaOH x Titre value x Volume made up x Equivalent weight of citric acid x 100}}{\text{Volume of sample x aliquot x 1000}}$$

3.5.5 Ash content (%)

The total ash content of citrus peel and pulp was determined as per the standard procedure of the Association of Analytical Chemists (A.O.A.C., 2005) using a muffle furnace. A known quantity of dried sample was placed in a previously weighed crucible. The crucible was placed in a muffle furnace and ignited at a temperature of 550-600℃ for 6 hours till the material was completely charred and turned into grey ash. The residue that remained in the crucible after cooling in a desiccator was weighed and expressed as a percentage.

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g) x 100}}{\text{Weight of the sample (g)}}$$

3.5.6 Reducing sugars (%)

The DNS method given by Miller (1959) was used for the determination of reducing sugar. A known volume of the sample was dissolved in 80% alcohol and vortexed. Then the samples were centrifuged at 3000 rpm for 20 minutes to extract the sugars. The collected supernatant was pooled and then kept in a water bath to evaporate the ethanol. 1 ml of distilled water was added to it in order to dissolve the sugars.

0.5 ml of the sample was pipetted out and the volume was made to 1 ml with distilled water. Dinitrosalicylic acid reagent was made by taking 100 ml of 2 N NaOH and dissolving 5 g of DNS. 250 ml of distilled water and 150 g of Rochelle salt were added to it and the final volume was made up to 500 ml. 3 ml of DNS reagent was added to each sample and then kept in a water bath for 5-10 minutes for the development of colour. The samples were allowed to cool down and the absorbance was measured at 540 nm by using a spectrophotometer. The amount of reducing sugar was expressed against the standard glucose equivalent using a standard regression equation calibration curve.

$$\text{Reducing sugar (\%)} = \frac{\text{Sugar value from graph x Total volume of extract}}{\text{Aliquot taken x Volume of the sample x 1000}}$$

3.5.7 Non-reducing sugars (%)

For the determination of non-reducing sugars, the sample is first hydrolysed with sulphuric acid to convert into reducing sugars and then determined by the DNS method given by Miller (1959) (Thimmiah, 1999).1 ml of extract was pipetted out in a test tube and 1 ml of 1 N H_2SO_4 was added to it. The mixture was heated at 49℃ for 30 minutes in order to hydrolyse it. After cooling the samples, 1 drop of methyl indicator was added to it. 1 N NaOH was added to the mixture dropwise in order to neutralise the acidity. 0.5 ml of the sample was pipetted out and then the volume was increased to 1 ml using distilled water. Each sample received 3 ml of DNS reagent and was placed in a water bath for approximately 5-10 minutes to allow the colour to develop. The samples were brought down to room temperature before taking a spectrophotometric reading at 540 nm. Using a typical regression equation calibration curve, the amount of non-reducing sugar was represented against the standard glucose equivalent.

$$\text{Non-reducing sugar (\%)} = \frac{\text{Sugar value from graph x Total volume of extract}}{\text{Aliquot taken x Volume of the sample x 1000}}$$

3.5.8 Total sugar (%)

Total sugar was calculated by adding the values of reducing and non-reducing sugar (Thimmiah, 1999).

3.5.9 Crude protein (%)

The quantitative estimation of crude protein was carried out by the Micro-Kjeldahl method as per A.O.A.C (2005). The peel and the pulp were initially dried and ground. 0.5 g of sample was taken and digested with the help of the digestion mixture made of $CuSO_4$ and K_2SO_4 in a ratio of 1:3, and 10 ml of concentrated Sulphuric acid. The sample was digested until colourless. After cooling, the sample was distilled with 40% NaOH. The distillation process released ammonia, which was recovered as ammonium borate in a 4% boric acid solution. A mixed indicator was prepared by mixing 0.2% alcoholic solution of methyl red (1 part) with 0.2% alcoholic solution of bromocresol green (5 part). Titration with 0.1N concentration H_2SO_4 was performed on the residue after adding mixed indicator. To

calculate crude protein content, the obtained value was multiplied by 6.25

$$\% \text{ Nitrogen} = \frac{14 \times (V-V1) \times X \times 100}{100 \times W}$$

% Crude Protein= % Nitrogen x 6.25

Where,

W= weight of the sample

V= Volume of H_2SO_4 required to neutralise ammonia

V1= Volume of H_2SO_4 used for blank

X= Strength of H_2SO_4

3.5.10 Organic acids (mg/100 ml)

Organic acid extraction was performed by utilizing the method given by Ergonul *et al.* (2010) with minor modifications. 1 ml of fresh fruit juice was extracted using a 1 ml 75:25 (V/V) water-methanol combination, followed by 30 minutes of centrifugation at 3500 rpm at room temperature. The supernatant was separated in a tube, and the extraction procedure was performed three times more. Before putting the extract into the UHPLC system, it was further filtered via a 2-micron Nylon filter. To create a standard OA calibration curve, standard stock solutions of 10 mM of each organic acid were utilised in order to prepare working solutions of different concentrations. The stock solution and its dilution were filtered through 0.2 m Nylon and kept at 4℃ in the dark.

To measure Organic acids, a Thermo Fisher Scientific UltiMate 3000 UHPLC System equipped with a Dionex-3000 photodiode array detector (PDA) (set at 210 nm) is utilized. 10 µl of the sample was injected into the injector, and organic acid separation was accomplished in a Syncronis C18 column (5m, 2.1 150 mm, Thermo Fisher) using 50mM Phosphate buffer (pH 2.8 adjusted with Phosphoric acid) as a mobile phase at 30 C for 6 minutes at a flow rate of 0.6 ml per min in isocratic mode. Individual peaks were measured and identified by comparing retention times to standard peaks.

3.5.11 Water-soluble vitamins (mg/100 ml)

Water soluble vitamins were analysed as per the methodology proposed by Wazed *et al.* (2022) with slight modifications. A UHPLC equipment coupled with a PDA (Photo Diode Array) detector was used to quantify water-soluble vitamins. V Sigma vitamin standards of

analytical grade were obtained from Sigma and combined in 10ml of HPLC water to achieve a final concentration of 1000 µg/ml. The vitamin B_9 or folic acid was first dissolved in 10 µL of 8 M KOH before being diluted in water up to a final volume of 10 ml. Dilution of the stock solution was done to make working solutions with varying concentrations (10-100 µg/ml). 10 µl of the working solution was injected into the UHPLC system to match the retention time of the unknown chemicals and create a standard calibration curve to measure the numerous water-soluble vitamins contained in the sample.

In order to prepare samples for Vitamin B analysis, they were first weighed to 10 g, homogenised with the help of mortar and pestle, and put in conical flasks containing 25 ml of extraction solution. The extraction solution was produced by combining 50 ml of acetonitrile with 10 ml of glacial acetic acid and the volume was ultimately made up to 1 litre with double distilled water. Samples were shaken in water.

For 40 minutes, samples were shaken in a water bath at a temperature of 70°C. The Samples were cooled, gravity filtered and mixed with extraction solution to make a final solution volume of 50 ml. Samples were filtered using 0.45 µm filter tips and with the help of an autosampler sampler aliquots of 20 µl were drawn from the solution and injected into the HPLC.

Water-soluble vitamins were separated by injecting 10 µl of extracted sample and standard solution into a Syncronis C18 column (5m, 2.1 150 mm, Thermo Fisher) UHPLC column linked to a Photodiode array detector (Model: Dionex UltiMate 3000) set to 210 nm. The UHPLC equipment was operated in isocratic mode with acetonitrile as solvent A and 10mM potassium dihydrogen phosphate solution (pH 2.5) as solvent B at a flow rate of 1.0ml/min for 5 mins. Individual vitamin peaks were also detected by comparing retention times and quantified by constructing the standard linear regression calibration equation by measuring the area of the standard.

3.5.12 Mineral analysis (mg/100 g)

The mineral solution was made in accordance with the A.O.A.C procedure (1970). Ash was dissolved in HCL in a ratio of 1:1 and evaporated to dryness over a water bath. After that, 4 ml water and 2 ml distilled water were added and warmed and the solution was filtered using filter paper. This solution was made up to 100 ml with distilled water in a volumetric flask. The solution was analysed in an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrument.

3.5.13 Essential oil content (%)

The sample of fresh and ambient fruit peels was subjected to hydrodistillation using a Clevenger apparatus. The essential oil was collected and the yield was calculated by the following formula:

$$\text{Oil (\%)} = \frac{\text{Volume of oil extracted} \times 100}{\text{Sample weight}}$$

3.5.14 Major volatile compounds in essential oil (Relative %)

Analysis of the essential oil was carried out by Triple Quadrupole Gas-Chromatography Mass-Spectroscopy/ Mass-Spectroscopy (TQGC-MS/MS) according to the procedure given by Mohammed *et al.* (2021). The gas chromatography-mass spectrometry instrument used to perform the analysis of the peel essential oil and has the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Triple Quadrupole Mass Spectrometer). A TG-5MS column (30 m 0.25 mm i.d., 0.25 m film thickness) was fitted with the GC-MS system. The analysis was performed with helium as the carrier gas, at a flow rate of 1.0 ml/min and a split ratio of 1:10. The temperature program used was as follows: starting at 60 °C for 1 minute, then increasing at a rate of 3.0 °C/min until reaching 240 °C, and held for 1 minute. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were injected. Using a spectral range of m/z 40-450, mass spectra were produced by electron ionisation (EI) at an energy of 70 eV. Most of the compounds were identified through the use of the analytical method, specifically by analyzing their mass spectra. This involved comparing the spectra to those of authentic chemicals found in both the Wiley spectral library collection and the NSIT library.

3.6 Statistical analysis

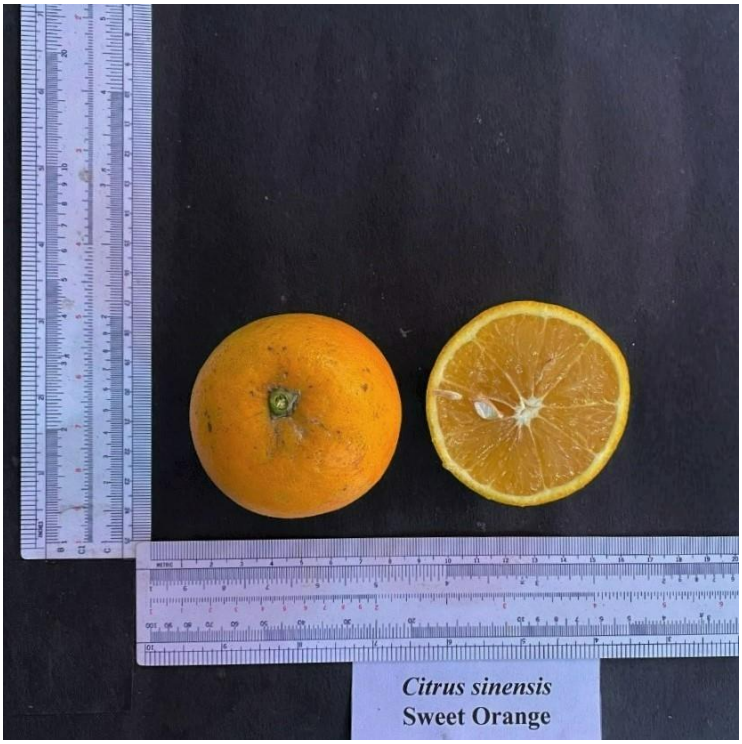
Statistical analysis was carried out with the help of IBM SPSS software.

PHOTOGRAPHS OF SAMPLES USED IN THE STUDY

SPECIMEN 1

SPECIMEN 2

SPECIMEN 3



SPECIMEN 4

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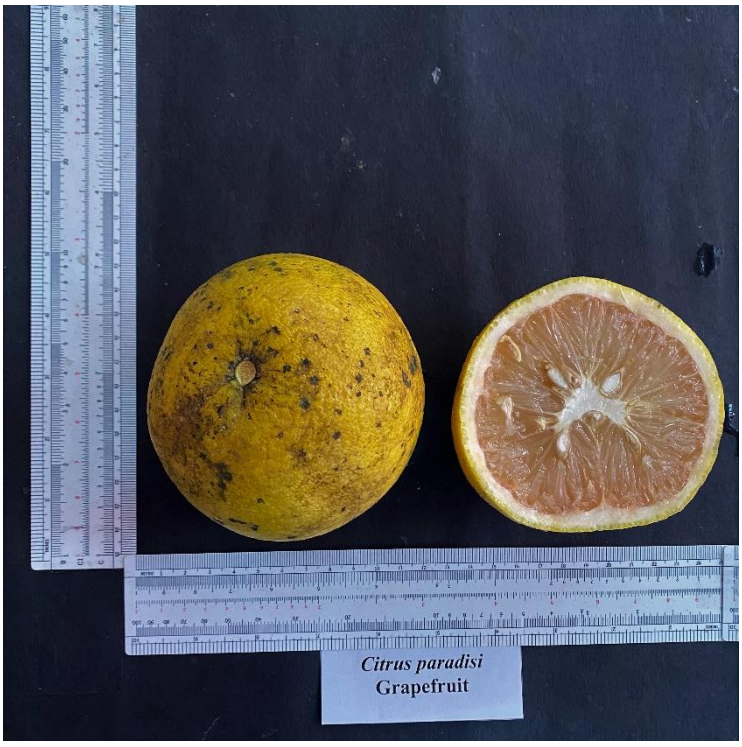

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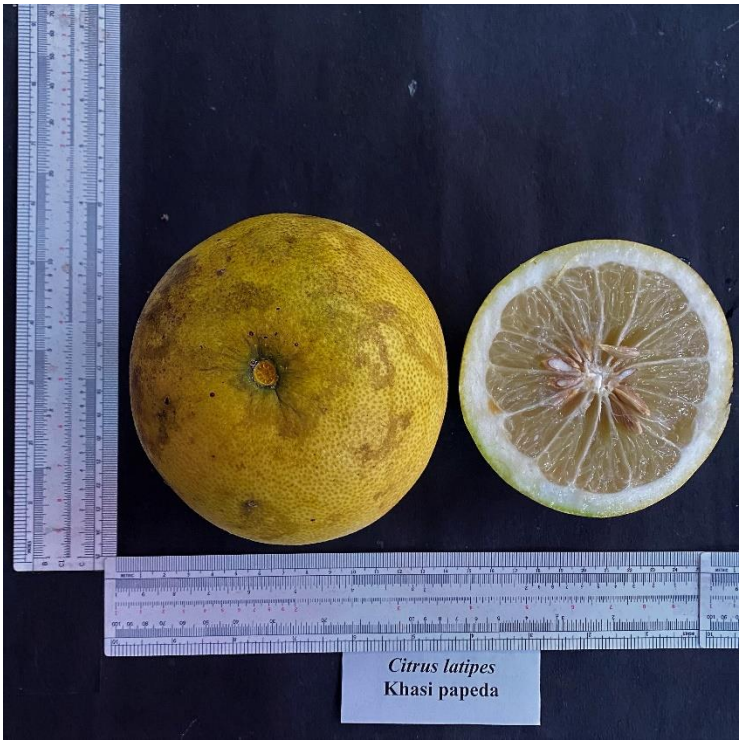
SPECIMEN 9



SPECIMEN 10

Experimental Findings… ✍

CHAPTER IV

EXPERIMENTAL FINDINGS

4.1 Morphological parameters

4.1.1 Fruit weight (g)

The data presented in Table 4.1B reveals that the maximum fruit weight was observed in Pummelo (826.4 g) while the lowest fruit weight was observed in Rangpur lime (50.37 g). The average fruit weight of the cultivars was 276.112 g. The results were found to be statistically significant at $p<0.05$. The statistically similar cultivars are grouped together using the same superscript as represented in the table.

4.1.2 Fruit length (cm)

Fruit length showed significant variation (Table 4.1B). Pummelo had the longest mean fruit length (14.57 cm), while the shortest fruit length was observed in Khasi mandarin (5.77 cm). The mean fruit length was recorded at 8.92 cm. It was determined that the results were statistically significant at $p< 0.05$. The statistically similar cultivars are grouped together using the same superscript as represented in the table.

4.1.3 Fruit breadth (cm)

Significant variation was observed in fruit breadth among genotypes (Table 4.1B). The maximum mean fruit breadth was observed in Pummelo (15.28 cm) which was significantly higher than all other genotypes. The minimum mean fruit breadth was observed in Rangpur lime (5.57 cm). The mean fruit breadth was recorded at 8.55 cm. It was determined that the results were statistically significant at $p< 0.05$. Utilising the same superscript, the statistically similar cultivars have been grouped together as represented in the table.

4.1.4 Number of segments

The data presented in Table 4.1B reveals that Pummelo was found to have the maximum number of segments (14.33) while Sweet Orange and Rangpur lime were found to have the minimum number of segments (9.67). The mean number of segments in fruits was recorded at 11.33. The results were found to be statistically significant at $p<0.05$. The statistically similar cultivars are grouped together using the same superscript as represented in the table.

4.1.5 Fruit rind thickness (mm)

The rind thickness of fruits varied from species to species and the maximum was recorded in Pummelo (16.10 mm) while the minimum was recorded in Cleopetra mandarin (1.23 mm). The average fruit rind thickness was recorded at 6.4 mm. The results were reported to be statistically significant at $p<0.05$. The statistically comparable genotypes have been grouped together using the same superscript as represented in Table 4.1B.

4.1.6 Juice content (%)

Results shown in Table 4.1B represent the juice content in *Citrus* fruits. The percentage of juice was found to be maximum in Sour orange (50.84%) and minimum in Khasi papeda (14.95%). The average juice content was found to be 36.49%. All the germplasms are found different statistically and are of significance.

4.1.7 Fruit shape

Spheroid fruit shape was observed in Rangpur lime, Sour orange, Sweet orange, Khasi mandarin, Cleopetra mandarin, Pummelo, Grapefruit, Kaffir lime and Khasi papeda while ellipsoid fruit shape was observed in Bira jora (Table 4.1A)

4.1.8 Shape of fruit base

The shape of the fruit base was observed as convex in Rangpur lime, Sour orange, Sweet orange, Cleopatra mandarin and Pummelo, as truncate in Khasi mandarin, as concave collard in Bira jora, as necked in Kaffir lime and as concave in Grapefruit and Khasi papeda (Table 4.1A).

4.1.9 Shape of fruit apex

Rounded fruit apex was observed in Rangpur lime, Sour orange, Sweet orange, Grapefruit, Kaffir lime and Khasi papeda. While in Khasi mandarin, Cleopatra mandarin and Pummelo truncate fruit apex was observed. Bira jora was found to have mammiform fruit apex. (Table 4.1A)

4.1.10 Fruit skin (epicarp colour)

The fruit skin (epicarp colour) was recorded as orange in Sweet orange, Sour orange, Khasi mandarin and Cleopatra mandarin. Green fruit skin colour was recorded in Kaffir lime while Bira jora and Khasi papeda were found to have yellow skin colour. Orange-green, yellow-green and dark yellow fruit skin colours were recorded in Rangpur lime, Pummelo and Grapefruit respectively (Table 4.1A).

4.1.11 Fruit surface texture

The fruit surface texture was observed as pitted in Rangpur lime and Sweet orange, bumpy in Bira jora and Kaffir lime, rough in Sour orange, Cleopatra mandarin and Grapefruit, smooth in Khasi mandarin and papillate in Pummelo and Khasi papeda. (Table 4.1A).

4.1.12 Albedo colour

The albedo colour was recorded as white in all genotypes except Pummelo where it was recorded as pink (Table 4.1A).

4.1.13 Adherence of albedo

Adherence of mesocarp to endocarp was recorded as weak in Rangpur lime, Bira jora, Khasi mandarin, Cleopatra mandarin and as strong in Sour orange, Sweet orange, Pummelo, Grapefruit, Kaffir lime and Khasi papeda. (Table 4.1A)

4.1.14 Fruit axis

Hollow fruit axis was observed in Khasi mandarin, Cleopatra mandarin, Grapefruit and Khasi papeda. Semi-hollow fruit axis was observed in Rangpur lime and Pummelo. Bira jora, Sour orange, Sweet orange and Kaffir lime were found to have solid fruit axis. (Table 4.1A)

4.1.15 Pulp colour

Pulp colour was observed as orange in Rangpur lime, Sweet orange and Khasi mandarin, as yellow in Sour orange, Cleopetra mandarin and Kaffir lime, as pink in Pummelo and Grapefruit and as white in Bira jora and Khasi papeda (Table 4.1A)

4.1.16 Pulp colour intensity

Pulp colour intensity was perceived as dark for Rangpur lime, Bira jora, Sour orange, Khasi mandarin, Cleopetra mandarin and Khasi papeda. Light intensity of pulp colour was recorded in Sweet orange, Pummelo, Grapefruit and Kaffir lime. (Table 4.1A)

4.1.17 Seeds per fruit

The seeds per fruit varied from species to species and the mean maximum number was recorded in Pummelo (47.67) while the mean minimum was recorded in Cleopetra mandarin (8.66). The average number of seed count was 21.73. The mean fruit length was recorded at 8.92 cm. It was determined that the results were statistically significant at $p< 0.05$. The statistically similar cultivars are grouped together using the same superscript as represented in Table 4. 1B.

4.1.18 Seed shape

Ovoid seed shape was observed in Rangpur lime, Bira jora, Sour orange, Cleopetra mandarin and Grapefruit. Clavate seed shape was recorded in Sweet orange and Khasi mandarin while semi-deltoid shape was recorded in Kaffir lime and Pummelo. Cuneiform seed shape was found to be present in Khasi papeda. (Table 4.1A)

4.1.19 Seed colour

Seed colour was observed as cream in all the genotypes except Bira jora where creamy white colour was observed and Grapefruit where white seed colour was observed. (Table 4.1A)

4.2 Biochemical Characteristics

4.2.1 Moisture content (%)

Among the peels of different genotypes, the highest moisture content was present in Rangpur lime peel (83.32%) and the lowest was present in Khasi mandarin peel (70.20%). Among the pulps of different genotypes, Rangpur lime had the highest moisture content (92.51%) while Kaffir lime had the lowest (84.30%). The results were found to be statistically significant at $p<0.05$. The statistically similar cultivars are grouped together using the same superscript as represented in Table 4.2.

4.2.2 Juice pH

It is evident from Table 4.2 that the mean values for pH ranged from 2.60 to 3.90 and was maximum in Khasi mandarin (3.90) and minimum in Rangpur lime (2.60). The statistically similar cultivars have been grouped using the same superscript as shown in Table 4.2. The results were determined to be statistically significant at $p<0.05$

4.2.3 Total soluble solids (TSS) (°Brix)

The mean values for TSS content ranged from 5.30 °Brix to 11.40 °Brix (Table 4.2). Among the different genotypes, Sweet orange had the highest TSS (11.40 °Brix) whereas Bira jora and Kaffir lime had the lowest (5.30 °Brix). The results were found to be statistically significant at $p<0.05$. The statistically similar cultivars are grouped together using the same superscript as represented in the table.

4.2.4 Titratable acidity (%)

The mean values for titratable acidity percentage ranged from 0.97% to 4.71%. Among the genotypes, Sweet orange recorded the lowest titratable acidity percentage (0.97%) whereas Kaffir lime recorded the highest titratable acidity percentage (4.71%). Cultivars that are statistically similar have been categorized with the same superscript, as demonstrated in

Table 4.2. The findings were deemed statistically significant with a p-value of < 0.05.

4.2.5 Ash content (%)

The mean values for ash content ranged from 2.533% to 5.690% in peel and from 2.833% to 5.963% in pulp. Among the peels of different genotypes, the highest ash % was found in Sour orange (5.69 %) and the lowest was found in Kaffir lime (2.53%). Among the pulps, the highest ash % was revealed in Rangpur lime (5.963%) and the lowest ash % was revealed in Khasi mandarin (2.833%). Cultivars that were found to be statistically similar have been grouped together and labelled with the same superscript in Table 4.3. The results were deemed statistically significant at $p< 0.05$.

4.2.6 Reducing Sugars (%)

The data in Table shows that the reducing sugar in juice among the different germplasm ranged from 2.06% to 6.72%. The highest reducing sugar was present in Khasi mandarin (6.72%) and the lowest was present in Bira Jora (2.061%). The results were found to be statistically significant at $p<0.05$. The cultivars that are statistically comparable to one another are grouped together by using the same superscript (Table 4.3).

4.2.7 Non-reducing sugars (%)

The non-reducing sugar in juice among the different genotypes of citrus ranged from 0.48% to 4.15%. The highest amount of non-reducing sugars was present in Sweet orange (4.145%) and the lowest amount was present in Bira Jora (0.48%). The results were found to be statistically significant at $p<0.05$. The statistically similar cultivars are grouped together using the same superscript as represented in Table 4.3.

4.2.8 Total sugar (%)

It is observed from Table 4.10 that the total sugar percentage in the juice was maximum in Khasi Mandarin (9.36%) and minimum in Bira Jora (2.54%). The results were found to be statistically significant at $p<0.05$. Using the same superscript, the statistically comparable cultivars have been grouped together (Table 4.3).

4.2.9 Crude protein (%)

The highest and lowest protein content among the peels was evident in Bira Jora (13.83%) and Grapefruit (5.6%) respectively. Meanwhile, among the pulps, the highest and lowest protein content was found in Sour orange (9.16%) and Pummelo (5.6%) respectively. The statistically comparable cultivars have been grouped together with identical superscripts in Table 4.3, and the statistical significance of the results was confirmed at $p<0.05$.

4.2.10 Organic acids (mg/100 ml)

Table 4.4 represents the organic acid profile in *Citrus* cultivars. The organic acid profile estimation of *Citrus* juice was carried out with the help of UHPLC. Seven organic acids were quantified namely oxalic acid, tartaric acid, malic acid, ascorbic acid, citric acid, fumaric acid and succinic acid. It was revealed that the average value of citric acid was the highest among all the acids. The highest citric acid was found in Rangpur lime (1807.89 mg/100 ml) and the lowest in Khasi papeda (24.35 mg/100 ml). Oxalic acid was highest in Bira Jora (30.83 mg/100 ml) and lowest in Cleopetra mandarin (7.53 mg/100 ml). The highest concentration of ascorbic acid was detected in Sweet orange (297.09 mg/100 g) and the lowest concentration was detected in Khasi papeda (9.66 mg/100 g). Similarly, tartaric acid was highest in Khasi papeda (171.81 mg/100 ml) and lowest in Rangpur lime (46.27 mg/100 ml). Tartaric acid was not detected in grapefruit. The highest and lowest content of malic acid was found in Khasi mandarin (623.64 mg/100 ml) and Pummelo respectively (16.98 mg/100 ml). The fumaric acid concentration was maximum in Sour orange (118.78 mg/100 ml) and minimum in Grapefruit (1.71 mg/100 ml). Fumaric acid was not detected in Bira Jora. The highest succinic acid concentration was observed in Kaffir lime (637.65 mg/100 ml) while the lowest was observed in Bira jora (57.97 mg/100 ml). Succinic acid was not detected in Khasi mandarin, Pummelo, Rangpur lime and Sour orange.

4.2.11 Water-soluble vitamins (mg/100 ml)

The data presented in Table 4.5 reveals that the water-soluble vitamin content showed significant variation among the cultivars. Five water-soluble vitamins were quantified namely thiamine, niacin, pyridoxin, pantothenic acid and folic acid. The thiamine content was highest among all the vitamins and was found to be maximum in Rangpur lime (125.40 mg/100 ml) and minimum in Sour orange (7.77 mg/100 ml). The second most abundant vitamin present was pyridoxine which was highest in Kaffir lime (79.78 mg/100 ml) and lowest in Sweet orange (3.30 mg/100 ml). Pyridoxine was not detected in four of the cultivars namely Rangpur lime, Bira jora, Khasi mandarin and Khasi papeda. The niacin content was highest in Rangpur lime (26.26 mg/100 ml) and lowest in Khasi papeda (5.18 mg/100 ml). In Khasi mandarin, the pantothenic acid content was maximum (21.83 mg/100 ml) and Rangpur lime was found to have the lowest concentration of pantothenic acid (1.77 mg/100 ml). The concentration of folic acid was highest in the genotype Bira jora (0.52 mg/100 ml) and the lowest was found in the genotype Rangpur lime (0.11 mg/100 ml). Folic acid was not detected in Kaffir lime.

4.2.12 Mineral analysis (mg/100 g)

Results for the mineral content of citrus peel and juice are represented in Table 4.6 and Table 4.7. It was found that sodium content was highest in Kaffir lime among the peels and the pulps (158.11 mg/100 g and 22.91 mg/100 g respectively). Magnesium was highest in Sour orange (291.49 mg/100 g) among the peels and in Cleopetra mandarin (131.49 mg/100 ml) among the pulps studied. Potassium content was highest in Rangpur lime peel (39.51 mg/100 g) and in Bira jora pulp (19.24 mg/100 g). The highest calcium content was detected in Sour orange (297.27 mg/100 g) among the peels and in Bira jora (128.28 mg/100 g) among the pulps. The iron content was highest in Sour orange peel (32.15 mg/100 g) and in Rangpur lime pulp (41.46 mg/100 g). Sour orange peel had the highest concentration of copper (2.01 mg/100 g) and zinc (16.01 mg/100 g) whereas, among the pulp, Cleopetra mandarin (0.55 mg/100 mg) and Bira jora (3.18 mg/100 mg) had the highest concentration of copper and zinc respectively. Heavy elements such as cadmium and lead were quantified and it was revealed that they occur in trace amounts with cadmium having an average value of 0.004 mg/100 g in peels and 0.003 mg/100 g in pulps respectively and lead having an average value of 0.003 mg/100 g in peels and 0.001 in pulp respectively.

4.2.13 Essential oil content (%)

The essential oil content (%) of peels calculated on a fresh weight basis is represented in Table 4.8 The data reveals that the essential oil content averaged at 0.48%. The highest essential oil yield was produced Rangpur lime by (0.83%) followed by Bira Jora (0.77%). The lowest essential oil yield was produced by Cleopatra mandarin (0.26%).

4.2.14 Volatile compounds in essential oil (Relative %)

Table 4.9 represents the important volatile compounds in the *Citrus* essential oil and their relative %. The most abundant monoterpene hydrocarbon compound in all the genotypes was D-limonene which varies from 45.7% to 81.58% depending on the cultivars. The maximum D-limonene % was found in Kaffir lime and the minimum % was found in Rangpur lime. γ -terpinene was another major monoterpene hydrocarbon that was found in the range of 0.1% in Bira Jora to 16.97% in Rangpur lime. The next monoterpene compounds in abundance were α -pinene and β-pinene which were present in amounts ranging from 3.78% (in Rangpur lime) to 0.43% (in Khasi papeda) and 28.28% (in Khasi papeda) to 3.1% (in Pummelo) respectively. α -pinene was not detected in Sour orange, Cleopatra mandarin and Grapefruit. Monoterpenoids such as **α** -phellandrene was found up to 0.57% (Rangpur lime). The β-Ocimene percentage was found highest in Bira Jora (2.32%).

Monoterpene alcohols were also identified in the essential oil. α -terpineol which is a crucial monoterpene alcohol was highest in Bira Jora (28.06%). Linalool, another monoterpene alcohol was highest in Grapefruit (4.59%). The highest percentage of terpene-4-ol was identified in Rangpur lime (1.71%).

Certain unique compounds were exclusively isolated in only certain *Citrus* species such as camphene (0.23%) in Rangpur lime, geraniol (0.71%) in Khasi Papeda and cis- α -bisabolene (0.42%) in Bira Jora. N- decanal was identified in only Sour orange (0.37%) and Rangpur lime (0.21%). The unique compounds in Sweet Orange were myrcene (0.17%) and nerol (0.06%) which were not identified in any other *Citrus* species under the study. Cis- α -bergamotene (0.21%) was identified in only Bira jora (0.21%) and Sour orange (0.17%). 2-carene was detected in grapefruit only and 3-carene in Sweet orange, Cleopatra mandarin and Sour orange. Thymol was identified in very low concentrations in Sweet orange (0.38%) and Eucalyptol was a unique compound that was present in Kaffir lime (3.86%).

Table: 4. 1A: Morphological Characteristics of the *Citrus* germplasm – Major Qualitative Characteristics												
Cultivars	*Fruit shape*	*Shape of fruit base*	*Shape of fruit apex*	*Fruit skin (epicarp) colour*	*Fruit surface texture*	*Albedo colour*	*Adherence of albedo*	*Fruit axis*	*Pulp colour*	*Pulp colour intensity*	*Seed shape*	*Seed colour*
Rangpur Lime	Spheroid	Convex	Rounded	Orange-green	Pitted	White	Weak	Semi-hollow	Orange	Dark	Ovoid	Cream
Bira Jora	Ellipsoid	Concave collard	Mammiform	Yellow	Bumpy	White	Weak	Solid	White	Dark	Ovoid	Creamy white
Sour orange	Spheroid	Convex	Rounded	Orange	Rough	White	Strong	Solid	Yellow	Dark	Ovoid	Cream
Sweet orange	Spheroid	Convex	Rounded	Orange	Pitted	White	Strong	Solid	Orange	Light	Clavate	Cream
Khasi mandarin	Spheroid	Truncate	Truncate	Orange	Smooth	White	Weak	Hollow	Orange	Dark	Clavate	Cream
Cleopatra mandarin	Spheroid	Convex	Truncate	Orange	Rough	White	Weak	Hollow	Yellow	Dark	Ovoid	Cream
Pummelo	Spheroid	Convex	Truncate	Yellow-green	Papillate	Pink	Strong	Semi-hollow	Pink	Light	Semi deltoid	Cream
Grapefruit	Spheroid	Concave	Rounded	Dark yellow	Rough	White	Strong	Hollow	Pink	Light	Ovoid	White
Kaffir lime	Spheroid	Necked	Rounded	Green	Bumpy	White	Strong	Solid	Yellow	Light	Semi deltoid	Cream
Khasi papeda	Spheroid	Concave	Rounded	Yellow	Papillate	White	Strong	Hollow	White	Dark	Cuneiform	Cream

Table: 4. 1B: Morphological Characteristics of the *Citrus* germplasm – Major Quantitative Characteristics

Cultivars	*Fruit weight(g)*	*Fruit length (cm)*	*Fruit breadth (cm)*	*Number of segments*	*Fruit rind thickness (mm)*	*Juice content (%)*	*Seeds per fruit*
Rangpur Lime	50.37 ± 2.64^{f}	6.40 ± 0.50^{d}	5.57 ± 0.32^{d}	9.67 ± 0.58^{c}	2.20 ± 0.26^{f}	40.19 ± 3.38^{bcde}	9.67 ± 1.53^{ef}
Bira Jora	166.80 ± 7.07^{d}	14.13 ± 0.57^{a}	9.63 ± 0.35^{c}	11.67 ± 0.58^{bc}	13.20 ± 0.60^{b}	33.01 ± 3.16^{e}	10.67 ± 1.16^{ef}
Sour orange	122.40 ± 8.17^{def}	7.10 ± 0.36^{d}	7.10 ± 0.3^{d}	10.67 ± 0.58^{c}	5.50 ± 0.50^{de}	50.84 ± 3.23^{a}	17.33 ± 1.53^{cd}
Sweet orange	150.00 ± 9.10^{d}	6.27 ± 0.20^{d}	6.47 ± 0.45^{d}	9.67 ± 0.58^{c}	4.47 ± 0.64^{e}	36.11 ± 1.29^{cde}	15.67 ± 2.08^{cde}
Khasi mandarin	133.80 ± 8.55^{def}	5.77 ± 0.57^{d}	6.10 ± 0.36^{d}	10.33 ± 0.58^{c}	1.73 ± 0.12^{f}	45.57 ± 2.70^{ab}	22.00 ± 2.0^{c}
Cleopetra mandarin	143.60 ± 7.95^{de}	6.73 ± 0.15^{d}	6.03 ± 0.21^{d}	11.00 ± 1.00^{bc}	1.23 ± 0.06^{f}	41.48 ± 2.76^{bcd}	8.67 ± 0.58^{f}
Pummelo	826.40 ± 76.56^{a}	14.57 ± 1.20^{a}	15.28 ± 0.95^{a}	14.33 ± 0.58^{a}	16.10 ± 0.53^{a}	23.51 ± 0.76^{f}	47.67 ± 2.52^{a}
Grapefruit	467.50 ± 11.05^{c}	9.10 ± 0.53^{c}	10.17 ± 1.33^{c}	13.33 ± 0.58^{ab}	6.37 ± 0.058^{d}	35.76 ± 2.83^{de}	30.33 ± 4.73^{b}
Kaffir lime	68.30 ± 2.46^{ef}	7.13 ± 0.40^{d}	6.50 ± 0.30^{d}	11.00 ± 1.00^{bc}	5.53 ± 0.15^{de}	43.48 ± 2.89^{abc}	14.00 ± 1.00^{def}
Khasi papeda	632.00 ± 17.25^{b}	12.00 ± 0.40^{b}	12.61 ± 0.38^{b}	11.67 ± 1.53^{bc}	7.80 ± 0.26^{c}	14.95 ± 0.94^{g}	41.33 ± 1.16^{a}
S. ED (±)	12.14	0.26	0.28	0.38	0.18	1.21	1.01
CD (p=0.05)	20.94	0.46	0.49	0.66	0.32	2.09	1.74

N.B: *Values are mean (n=3) ± S.D. Values with different superscript letters (a to f) in the same column are statistically different ($p<0.05$)*

Table: 4. 2: Moisture content (%), Juice pH, TSS (°Brix) and Titratable acidity (%) of the *Citrus* germplasm

Cultivars	*Moisture content (%)*		*Juice pH*	*TSS (°Brix) of juice*	*Titratable acidity (%) of juice*
	Pulp	*Peel*			
Rangpur Lime	92.51 ± 0.97^{a}	83.32 ± 1.33^{a}	2.60 ± 0.05^{f}	5.47 ± 0.15^{f}	4.58 ± 0.03^{b}
Bira Jora	87.21 ± 1.17^{bcde}	75.80 ± 0.89^{b}	2.84 ± 0.03^{e}	5.30 ± 0.10^{f}	4.31 ± 0.04^{c}
Sour orange	91.47 ± 0.58^{ab}	82.28 ± 2.65^{a}	2.69 ± 0.03^{ef}	6.93 ± 0.058^{e}	4.25 ± 0.05^{c}
Sweet orange	88.37 ± 3.19^{abcde}	74.22 ± 1.44^{bc}	3.89 ± 0.86^{a}	11.4 ± 0.10^{a}	0.97 ± 0.05^{i}
Khasi mandarin	88.54 ± 1.30^{abcde}	70.20 ± 1.43^{c}	3.90 ± 0.01^{a}	10.53 ± 0.15^{b}	1.23 ± 0.08^{h}
Cleopetra mandarin	86.45 ± 1.42^{cde}	76.49 ± 2.03^{b}	3.35 ± 0.15^{c}	11.37 ± 0.15^{a}	3.07 ± 0.07^{e}
Pummelo	85.28 ± 2.17^{de}	76.16 ± 1.06^{b}	3.69 ± 0.05^{b}	8.77 ± 0.15^{c}	2.20 ± 0.05^{f}
Grapefruit	89.68 ± 0.95^{abcd}	70.81 ± 1.29^{c}	3.38 ± 0.07^{c}	8.27 ± 0.06^{d}	1.98 ± 0.07^{g}
Kaffir lime	84.30 ± 1.57^{e}	73.84 ± 1.26^{bc}	3.80 ± 0.01^{ab}	5.30 ± 0.10^{f}	4.71 ± 0.14^{a}
Khasi papeda	90.83 ± 0.67^{abc}	72.73 ± 1.28^{bc}	3.07 ± 0.04^{d}	7.13 ± 0.25^{e}	3.98 ± 0.07^{d}
S. ED (±)	0.746	0.728	0.030	0.066	0.033
CD (p=0.05)	1.286	1.256	0.052	0.113	0.057

N.B: Values are mean (n=3) ± S.D. Values with different superscript letters (a to g) in the same column are statistically different (p<0.05)

Table: 4.3: Ash content (%), Sugar (%) of the *Citrus* germplasm and Crude protein (% in dry weight basis)

Cultivars	*Ash content (%)*		*Sugar (%) in juice*			*Crude protein content (%)*	
	Pulp	*Peel*	*Reducing sugar*	*Non reducing sugar*	*Total sugar*	*Pulp*	*Peel*
Rangpur Lime	5.96 ± 0.03^{a}	5.50 ± 0.01^{a}	2.82 ± 0.09^{e}	2.54 ± 0.06^{de}	5.36 ± 0.09^{e}	6.80 ± 0.28^{e}	10.44 ± 0.36^{c}
Bira Jora	4.41 ± 0.09^{c}	5.12 ± 0.10^{b}	2.06 ± 0.19^{f}	0.48 ± 0.05^{h}	2.54 ± 0.17^{g}	7.76 ± 0.36^{cd}	13.83 ± 0.35^{a}
Sour orange	5.44 ± 0.18^{b}	5.69 ± 0.05^{a}	3.35 ± 0.08^{d}	2.27 ± 0.09^{f}	5.62 ± 0.16^{e}	9.16 ± 0.27^{a}	11.32 ± 0.27^{b}
Sweet orange	4.17 ± 0.15^{cd}	3.57 ± 0.06^{d}	4.99 ± 0.15^{c}	4.15 ± 0.07^{a}	9.13 ± 0.17^{ab}	8.52 ± 0.27^{abc}	7.35 ± 0.35^{e}
Khasi mandarin	2.83 ± 0.14^{f}	3.12 ± 0.11^{e}	6.72 ± 0.18^{a}	2.64 ± 0.12^{d}	9.36 ± 0.30^{a}	7.99 ± 0.27^{bcd}	7.35 ± 0.46^{e}
Cleopetra mandarin	3.99 ± 0.07^{d}	3.02 ± 0.03^{e}	5.66 ± 0.19^{b}	3.08 ± 0.07^{c}	8.74 ± 0.24^{b}	6.71 ± 0.36^{e}	8.05 ± 0.18^{e}
Pummelo	3.95 ± 0.13^{d}	4.07 ± 0.06^{c}	4.67 ± 0.13^{c}	3.43 ± 0.10^{b}	8.10 ± 0.10^{c}	5.60 ± 0.30^{f}	7.88 ± 0.27^{e}
Grapefruit	3.49 ± 0.02^{e}	3.60 ± 0.05^{d}	3.69 ± 0.12^{d}	2.80 ± 0.05^{d}	6.50 ± 0.16^{d}	7.53 ± 0.18^{de}	5.60 ± 0.18^{g}
Kaffir lime	4.16 ± 0.14^{cd}	2.53 ± 0.06^{f}	2.35 ± 0.20^{ef}	1.60 ± 0.05^{g}	3.95 ± 0.15^{f}	8.80 ± 0.27^{ab}	9.22 ± 0.27^{d}
Khasi papeda	4.27 ± 0.24^{cd}	3.99 ± 0.05^{c}	3.44 ± 0.12^{d}	2.42 ± 0.04^{ef}	5.87 ± 0.12^{e}	5.71 ± 0.27^{f}	6.48 ± 0.18^{f}
S. ED (±)	0.064	0.030	0.072	0.035	0.083	0.135	0.141
CD (p=0.05)	0.110	0.052	0.124	0.061	0.144	0.233	0.357

N.B: Values are mean (n=3) ± S.D. Values with different superscript letters (a to g) in the same column are statistically different ($p<0.05$)

Table: 4.4: Organic acid profile in juice of the *Citrus* germplasm

Cultivars	*Oxalic acid (mg/100 ml)*	*Tartaric acid (mg/100 ml)*	*Malic acid (mg/100 ml)*	*Ascorbic acid (mg/100 ml)*	*Citric acid (mg/100 ml)*	*Fumaric acid (mg/100 ml)*	*Succinic acid (mg/100 ml)*
Rangpur Lime	7.69	46.27	413.02	41.12	1807.89	96.03	N/A
Bira Jora	30.83	137.34	410.60	124.90	1122.18	N/A	57.97
Sour orange	13.78	102.46	268.70	39.22	1560.43	118.78	N/A
Sweet orange	8.68	71.86	539.12	297.09	553.03	32.45	493.00
Khasi mandarin	22.30	170.75	623.64	125.28	447.82	23.22	N/A
Cleopetra mandarin	7.53	118.73	83.50	197.37	775.34	50.76	487.74
Pummelo	11.67	113.07	16.98	134.48	18.41	48.62	N/A
Grapefruit	25.47	N/A	299.60	104.30	544.19	1.71	413.50
Kaffir lime	7.61	61.11	386.65	182.84	351.47	21.16	637.65
Khasi papeda	11.10	171.81	89.68	9.66	24.35	94.44	216.00

Table: 4. 5: Water-soluble vitamins in the juice of the *Citrus* germplasm					
Cultivars	*Thiamine (mg/100 ml)*	*Niacin (mg/100 ml)*	*Pyridoxine (mg/100 ml)*	*Pantothenic acid (mg/100 ml)*	*Folic acid (mg/100 ml)*
Rangpur Lime	125.40	26.26	N/A	1.77	0.11
Bira Jora	92.73	12.83	N/A	4.66	0.52
Sour orange	7.77	17.16	30.66	4.22	0.18
Sweet orange	73.15	10.49	3.30	4.52	0.40
Khasi mandarin	73.23	8.21	N/A	21.83	0.36
Cleopetra mandarin	28.43	16.17	17.59	14.59	0.20
Pummelo	38.15	8.91	38.50	3.90	0.33
Grapefruit	66.74	10.82	26.49	2.92	0.46
Kaffir lime	53.82	18.46	79.78	2.81	N/A
Khasi papeda	30.58	5.18	N/A	2.44	0.23

Table: 4. 6: Major minerals of the *Citrus* germplasm (fresh weight basis)

Cultivars	*Sodium (mg/100 g)*		*Magnesium (mg/100 g)*		*Potassium (mg/100 g)*		*Calcium (mg/100 g)*	
	Peel	*Pulp*	*Peel*	*Pulp*	*Peel*	*Pulp*	*Peel*	*Pulp*
Rangpur Lime	39.89	11.08	146.66	44.02	39.51	7.99	151.55	16.06
Bira Jora	30.29	17.15	53.76	84.17	10.57	19.24	67.41	128.38
Sour orange	25.35	10.30	291.49	71.53	34.03	10.47	297.27	36.14
Sweet orange	26.93	12.35	133.50	63.96	27.13	11.72	122.49	17.78
Khasi mandarin	40.86	12.95	237.46	69.01	17.75	7.04	159.84	31.75
Cleopetra mandarin	51.80	9.98	281.67	131.49	11.11	11.86	161.77	62.75
Pummelo	26.32	17.23	211.32	85.84	20.37	15.11	202.77	42.27
Grapefruit	21.60	11.86	89.20	52.81	20.54	10.31	104.69	14.86
Kaffir lime	158.11	22.91	180.51	74.03	7.98	7.59	116.20	30.44
Khasi papeda	30.30	17.49	160.95	21.44	23.06	3.29	123.51	4.89

Table: 4. 7: Minor minerals and heavy metals of the *Citrus* germplasm (fresh weight basis)

Cultivars	*Iron (mg/100 g)*		*Copper (mg/100 g)*		*Zinc (mg/100 g)*		*Cadmium (mg/100 g)*		*Lead (mg/100 g)*	
	Peel	*Pulp*	*Peel*	*Pulp*	*Peel*	*Pulp*	*Peel*	*Pulp*	*Peel*	*Pulp*
Rangpur Lime	7.48	41.46	0.89	0.20	2.69	1.61	0.003	0.001	0.001	0.001
Bira Jora	28.64	29.67	0.32	0.37	3.72	3.18	0.005	0.010	0.004	0.001
Sour orange	32.15	8.26	2.01	0.43	16.01	1.57	0.007	0.003	0.003	0.001
Sweet orange	6.40	4.17	0.52	0.40	1.72	1.06	0.005	0.002	0.005	0.004
Khasi mandarin	6.07	3.25	0.78	0.17	1.08	0.48	0.000	0.002	0.001	0.001
Cleopetra mandarin	18.05	4.84	0.47	0.55	2.10	1.75	0.005	0.004	0.003	0.001
Pummelo	6.98	4.01	0.45	0.35	0.85	0.54	0.002	0.001	0.001	0.001
Grapefruit	8.46	2.59	1.89	0.24	1.98	0.41	0.003	0.002	0.002	0.001
Kaffir lime	6.73	2.78	0.36	0.29	0.74	0.68	0.008	0.002	0.003	0.001
Khasi papeda	9.91	1.50	0.57	0.13	2.81	1.47	0.005	0.002	0.005	0.001

Table 4.8: Essential oil content (%) in Citrus peel (fresh weight basis)	
Cultivars	*Essential oil (%) in peels*
Rangpur Lime	0.83^{a}
Bira Jora	0.77^{a}
Sour orange	0.40^{cd}
Sweet orange	0.46^{bc}
Khasi mandarin	0.32^{def}
Cleopetra mandarin	0.26^{f}
Pummelo	0.29^{ef}
Grapefruit	0.52^{b}
Kaffir lime	0.37^{cde}
Khasi papeda	0.55^{b}
S. ED (±)	0.016
CD (p=0.05)	0.028

N.B: Values are mean (n=3) ± S.D. Values with different superscript letters (a to g) in the same column are statistically different (p<0.05)

Table: 4.9: Major volatile compounds found in the essential oil of the *Citrus* germplasm (Relative %).										
Compound	**Rangpur Lime**	**Bira Jora**	**Sour orange**	**Sweet orange**	**Khasi mandarin**	**Cleopetra mandarin**	**Pummelo**	**Grapefruit**	**Kaffir lime**	**Khasi papeda**
α -pinene	3.78	0.61	-	1.77	2.24	-	0.66	-	1.57	0.43
camphene	0.23	-	0.15	-	-	-	-	-	-	-
β-pinene	12.83	2.96	4.02	4.46	4.72	4.51	3.1	3.78	4.12	28.28
myrcene	-	-	-	0.17	-	-	-	-	-	-
α -phellandrene	0.57	0.11	-	-	0.11	0.16	-	0.11	0.15	0.08
α -terpinene	16.97	0.1	9.12	13.03	10.92	0.9	1.82	0.14	-	-
Linalool	-	0.27	0.32	1.37	0.36	3.06	1.04	4.59	0.76	1.25
D-limonene	**45.7**	**57.56**	**48.45**	**66.11**	**75.78**	**76.8**	**63.37**	**81.34**	**81.58**	**63.17**
nonanal	-	-	0.17	0.07	0.03	-	-	0.3	-	0.1
terpinen-4-ol	1.71	0.25	-	1.1	-	2.33	0.3	0.45	0.66	0.14
α -terpineol	-	28.06	-	3.04	0.61	0.97	-	0.43	1.41	-
n-decanal	0.21	-	0.37	-	-	-	-	-	-	-
nerol	-	-	-	0.06	-	-	-	-	-	-
geraniol	-	-	-	-	-	-	-	-	-	0.71
cis- α -bergamotene	-	0.21	0.17	-	-	-	-	-	-	-
β-bisabolene	-	0.28	-	-	-	-	-	-	-	-
o- cymene	1.15	-	-	1.87	1.1	-	0.17	-	0.18	-
β-Ocimene	0.40	2.32	1.62	0.18	-	0.41	0.17	-	-	0.12
endo-Borneol	0.24	0.1	-	-	-	-	-	-	-	-
β-longipinene	0.10	-	-	-	-	-	-	-	-	0.15
cis- α -Bisabolene	0.09	0.42	-	-	-	-	-	-	-	-
2-Carene	-	-	0.30	-	-	-	-	0.95	-	-
3- Carene	-	-	-	0.12	-	1.51	-	-	-	-
Thymol	-	-	-	0.38	-	-	-	-	-	-
Eucalyptol	-	-	-	-	-	-	-	-	3.86	-

Discussion… 

CHAPTER V

DISCUSSION

5.1 Morphological parameters

5.1.1 Fruit weight

The weight of the fruit is an important indicator of its quality and affects its market value and helps to plan packaging, transportation and market operations (Ifmalinda *et al.*, 2022). Weight is also one of the most important factors in understanding water and nutrient use efficiency, biomass composition, fruit consistency, identification of cultivars, determining crop values, and fruit acceptance by consumers (Ornelas-Paz *et al*., 2013). Significant variations were observed for fruit weight among the different genotypes, ranging from 50.37 g to 826.4 g and a mean of 276.112 g. Similar observations have been reported by Rabha *et al.* (2013) who found of range of 21.56 g to 712.53 g in fruit weight among 32 *Citrus* genotypes of Arunachal Pradesh. According to Rahman *et al.* (2003), the weight of the pummelo fruits varied from 718 to 2160 g which is not in conformity with the present findings.

The variation in fruit weight among *Citrus* genotypes might be due to the differential translocation of photosynthates from fully developed leaves to developing fruits (Singh *et al*., 2022). Furthermore, it had been reported that increased pulp tissue caused an increase in fruit weight which can be attributed to cell division during the early stages of fruit development, caused mainly by the thickness of the peel tissue (Dalal *et al*., 2013). The variation in fruit weight can be attributed to genotypic variations, environmental conditions and management practices (Basak *et al*., 2022; Gaikwad *et al*., 2018; Kayesh *et al.*, 2018; Zekri *et al.*, 2011).

5.1.2 Fruit length

The physical characteristics of fruit crops such as fruit length are the most crucial parameters to determine the appropriate standards for the design of grading, conveying, processing, and packaging systems (Tabatabaeefar and Rajabipour, 2005). Fruit length across the several genotypes showed significant variations with a range of 5.77 cm to 14.57 cm and a mean length of 8.92 cm. The present findings are in line with those of Rabha *et al.*

(2013) who observed a range of 3.02 cm to 12.3 cm among 32 *Citrus* genotypes of Arunachal Pradesh. The results are also similar to the findings of Gaikwad *et al.* (2018) who reported a range of 4.77 cm to 5.23 cm in rangpur lime genotypes and 6.20 to 108.05 cm in rough lemon genotypes of Maharashtra. The fruit length has a significant and positive correlation with the fruit weight (Herath *et al*., 2016). Usually, variations in fruit length among different genotypes are due to differences in genetic factors and micro-climate conditions. (Kayesh *et al*., 2018; Zekri *et al*., 2011).

5.1.3 Fruit breadth

Similar to fruit length, fruit breadth is also an important characteristic to design grading, conveying, processing, and packaging systems (Tabatabaeefar and Rajabipour, 2005). The data pertaining to fruit breadth reveals a significant variation among cultivars with a range of 5.57 cm to 15.28 cm and an average value of 8.55 cm. Similar findings were reported by Jaskani *et al.* (2006) who observed a breadth of 7.3 cm and 6.0 cm in bittersweet orange and sour orange respectively. The fruit breadths documented by Herath *et al.* (2016) are 4.05 cm in lime, 5.54 cm in lemon, 4.36 cm in mandarin, 9.9 cm in grapefruit and 14.2 cm in pummelo which are comparable with the present findings. The fruit weight and fruit breadth of *Citrus* fruits are found to be positively correlated with each other (Herath *et al*., 2016). Variations in genetic factors and climate could all contribute to the diversity in fruit breadth among genotypes (Kayesh *et al*., 2018; Zekri *et al*., 2011).

5.1.4 Number of segments

The taxonomy of *Citrus* relatives has been significantly influenced by the segment number in *Citrus* fruits (Tanaka, 1959; Swingle and Recee, 1967). The number of segments is an important characteristic for processing as the mechanically extracted juice contains segments which should be removed (Tisserat *et al.,* 1990). The number of segments ranged from 9.67 to 14.33 nos. with a mean of 11.33. This result is in line with the findings of Jaskani *et al.* (2016) who reported the number of segments to be 10.4 in sour orange and Rahman *et al.* (2003) who found it to be 11.28 in pummelo. Malik *et al*. (2006) reported the number of segments as 10.6 in *Citrus indica* and 14.2 in *Citrus macroptera* which is comparable to the present findings. The variation in the number of segments arises due to genetic factors and the size of the fruit (Liu *et al.,* 2005; Tisserat *et al*., 1990).

5.1.5 Fruit rind thickness

The fruit rind is crucial for preventing moisture loss, safeguarding the fruit from insects and other pests and minimising injuries including chilling injury (Nordby and

McDonald, 1990). The aroma that *Citrus* fruits emit and the flavours they are associated with are both characterized by the aroma-active volatile flavouring chemicals found in the rind or peel oils (Liu *et al*, 2012). A thicker peel reduces the likelihood of fruit splitting whereas easy-peeling fruit is a desirable feature for fresh consumption (Cronje *et al.*, 2013). The observation on fruit rind thickness reveals significant variation with a range of 1.23 mm to 16.10 mm among genotypes and an average of 6.2 mm. The results are in accordance with the findings reported by Sayed *et al.* (2006) and Akhter *et al.* (2009) who observed great variation in fruit rind thickness ranging from 1 to 2 mm, 2.5 to 5.0 mm and 4.3 to 27.5 mm in lime, lemon and jamir (*Citrus jambhiri*) accessions respectively. The genetic makeup of the various genotypes, tree age, fruit size and the various growing environments are responsible for the variation in rind thickness (Morgan *et al.*, 2005; Khalid *et al.*, 2012). Cohen (1976) noted the effect of higher potassium content on increased rind thickness.

5.1.6 Juice content (%)

The juice in fruits is found in sacs that are tightly packed, club-shaped and multicellular which are known as juice vesicles (Ladaniya, 2008). Though primarily water, the juice contains sugars (such as glucose, fructose, and sucrose), organic acids (such as malic, citric, and tartaric), fats, proteins, various volatile compounds and vitamins (Nagy *et al*., 1993). The juice content of a fruit is a crucial factor for juice processing industries. A significant difference in juice content (%) among the ten *Citrus* genotypes was observed. The juice content (%) varied from 14.95% to 50.84%. with an average percentage value of 36.49%. Similar reports were observed by Gaikwad *et al*. (2018) who found juice content ranging from 14.71% to 47.63% in Rough lemon and from 27.73% to 48.75% in Rangpur lime genotypes of Maharashtra, and by Singh (2010) who reported juice content ranging from 17.3% to 36.81% in Pummelo genotypes. Usually, the variation in juice content is caused by variations in the genetic makeup, growing region, fruit harvesting stage, irrigation and climate and method of juice extraction. (Wiegand *et al*., 1982; Castle *et al*., 2010)

5.1.7 Fruit shape

Fruit shape is an important characteristic for taxonomic classification and is also an indicator of the external quality of the fruit (Ladaniya, 2008). The shape of fruit can undergo change as they mature and that can be used as a characteristic to identify the harvest maturity in *Citrus* (Erkan and Dogan, 2019). Knowledge of fruit shapes is required in designing postharvest equipment such as handling, sorting, grading and packing systems (Khadivi-Khub, 2013). Two distinct types were recorded with respect to fruit shape. The genotype

Bira jora had an ellipsoid fruit shape and all other types had a spheroid fruit shape. The findings were in concurrence with the reports of Sayed *et al.* (2006) and Jaskani *et al.* (2006) who reported spheroid and ellipsoid shapes in lime, obolid, ellipsoid and ovoid shapes in lemon, ellipsoid fruit shape in bittersweet orange, spheroid shape in Yuma citrange and ellipsoid fruit shape in sour orange. The variation in fruit shape among different cultivar arises due to genetic differences and environmental factors (Kayesh *et al.*, 2018).

5.1.8 Shape of fruit base

The shape of the fruit base is an important morphological marker of *Citrus* fruits and is used as a parameter in taxonomy (Ladaniya, 2008). The fruit base shape showed significant variation with 5 genotypes showing a convex base shape, 1 genotype each showing a concave-collard shape, truncate shape and necked shape and 2 genotypes showing a concave fruit base shape. Gaikwad *et al.* (2018) and Sayed *et al.* (2006) also reported significant variations in the shape of the fruit base ranging from convex in Rangpur lime, convex in Galgal, concave, convex and truncate base in lime and concave collard, convex and necked in lemon. The variation in fruit base shape is contributed by genetic differences among the genotypes (Ladaniya, 2008).

5.1.9 Shape of fruit apex

The shape of the fruit apex is crucial for taxonomic classification and serves as an important parameter for characterization and identification (Ladaniya, 2008). Variation was seen in the shape of the fruit apex in the cultivars under study. Rounded fruit apex occurred in 6 genotypes, truncate fruit apex occurred in 3 genotypes and mammiform fruit apex occurred in 1 genotype. Variations in fruit apex were also reported by Malik *et al.* (2006), and Gaikwad *et al.* (2018) who observed truncate apex in *Citrus indica*, rounded apex in *Citrus macroptera*, mammiform and rounded apex in rangpur lime, truncate, mammiform and rounded apex in rough lemon and mammiform apex in galgal. The reason for the variation is due to differences in the genetic makeup of the cultivars (Ladaniya, 2008).

5.1.10 Fruit skin (epicarp colour)

Fruit skin colour is an important maturity index (Iqbal *et al.*, 2016). The epicarp colour is a biochemical aspect of the ripening process and is crucial for fruit quality (Tadeo *et al.*, 2008). Epicarp colour is also a factor in sorting and grading (Ladaniya, 2008). Fruit epicarp colour displayed considerable variations and the observations revealed that four genotypes had orange colour, two genotypes had yellow colour, one genotype had orange green colour, one had dark yellow colour and one had yellow-green colour. Variation in fruit skin (epicarp)

colour was also reported by Rahman *et al.* (2003), Singh and Singh (2006), where the fruit skin (epicarp) colour varied from orange, green, and yellow in rangpur lime, greenish-yellow, yellow, orange, reddish yellow. According to Koshita (2015), secondary metabolites including phenolic chemicals and terpenoids are responsible for determining the colour of fruits. The temperature has a significant influence on how *Citrus* fruit develops its typical rind colour. Environmental temperatures control both chlorophyll breakdown and synthesis of carotenoid and other fruit pigments influencing the colour break in *Citrus* that is dependent on changing pigment concentrations in the flavedo (Tadeo *et al*., 2008). Usually, the difference in fruit skin colour is due to genetic factors, harvest stage, microclimatic conditions and the position of the fruit on the tree (Stewart 1975; Zekri *et al.*, 2011; Erkan and Dogan, 2019).

5.1.11 Fruit surface texture

The fruit surface texture is an important morphological marker and an important factor for consumer acceptability. The fruit surface texture showed considerable variation among all the genotypes studied. The cultivars displayed rough (three genotypes), bumpy (two genotypes), pitted (two genotypes), smooth (one genotype) and papillate (two genotypes) texture of the surface. Similar variations were also observed by Jaskani *et al.* (2006) and Gaikwad *et al.* (2015), as they witnessed smooth, papillate, pitted bumpy and rough textures in Pummelo genotypes and rough textures bittersweet orange, Yuma citrange and Sour orange. The textural difference in the peel arises due to genotypic variations and due to ripening (Seymour *et al.*, 2002). The size and placement of oil glands in the flavedo, which may result in the development of small papillates or pits, as well as the possibility of a rough, wrinkly, bumpy, and ribbed surface, contribute to the variance in fruit surface texture (Hodgson, 1967).

5.1.12 Albedo colour

The albedo is the inner part of the fruit rind under the flavedo and contains pectins, cellulose, and flavonoids (Sandhu *et al*., 2012). Albedo is made up of the inner mesocarp, which is made up of parenchymatous cells with extensive air gaps (Scott and Baker, 1947). The albedo acts as an effective cushioning material against pressure and impact to fruits. (Ladaniya, 2008). The albedo colour was observed as white in all genotypes except Pummelo where it was recorded as pink. The findings are in line with those of Dey *et al.* (2016) and Jaskani *et al.* (2006) who also observed white albedo colour in bitter-sweet, white albedo colour in sour orange, and white and pink albedo colour in pummelo. The variation in albedo

colour arises due to genetic differences and environmental factors (Ladaniya, 2008; Singh *et al*, 2010). Different ratios of lycopene/b-carotene impact the colour of the albedo (Xu *et al*., 2006). Dey *et al.* (2016) stated that canopy position had an impact on the albedo colour of pummelo fruits

5.1.13 Adherence of albedo

The adherence of albedo to the pulp influences the extent of easy removal of the peel from the pulp or the peeling efficiency (Liu *et al.*, 2005). Pectin, cellulose and hemicellulose are responsible for the adherence of the fruit's skin (Whitaker, 1984). The cultivars which have weak peel-pulp adherence are easy to peel and have an intact colour and good appearance after peeling as compared to the fruits which have a strong adherence to the albedo (Liu *et al*, 2005). Thus, cultivars with weak albedo adherence are likely to be ideal for the catering industry. The findings of the present study reveal that six genotypes had shown strong adherence of albedo to the pulp while four genotypes had shown weak adherence of albedo to the pulp. Similar findings were reported by Jaskani *et al.* (2006) and Malik *et al.* (2006) who observed albedo adherence as medium to strong in Sour orange, medium in *Citrus* indica, and strong in *Citrus macroptera.* The variation in adherence of albedo to the pulp is due to genetic factors (Yu *et al.*, 2021).

5.1.14 Fruit axis

The edible pulp of mature *Citrus* fruit is made up of the segments that surround the central axis (Matheyambath *et al*., 2016). All *Citrus* fruits have a solid centre whilst they are still developing but in some of these fruits, the central axis may open up as they mature (Ladaniya, 2008). The fruit axis is a characteristic feature of *Citrus* species and is used as an important morphological marker. The observations pertaining to the fruit axis reveal that four of the genotypes exhibited hollow fruit axis. Semi-hollow fruit axis was observed in two of the genotypes while the remaining four genotypes exhibited a solid fruit axis. Similar irregularities in fruit axis were reported by Singh and Singh (2006), and Gaikwad *et al.* (2018) which varied in Rough lemon as hollow and semi-hollow, in Rangpur lime as hollow, semi-hollow and solid and in Galgal and Alemow as hollow. The variation in the fruit axis is due to differences in cultivar and maturity (Singh *et al.*, 2010; Sarkar, 2019).

5.1.15 Pulp colour

Appearance, including fruit pulp colour, is generally used as a selection criterion throughout the supply and consumer chain (Cebadera *et al*., 2019). Attractive internal colour in *Citrus* is usually an important prerequisite for processing (Tadeo *et al*., 2008). Pulp colour

varied among different genotypes as orange (three genotypes), yellow (three genotypes) pink (three genotypes) and white (two genotypes). Similar variations in *Citrus* pulp colour were also reported by Hassan *et al.* (2008) and Gaikwad *et al.* (2018) who documented orange colour in Rangpur lime, yellow colour in Rough lemon and white colour in Galgal and Alemow. According to Ajon (1943), several *Citrus* fruits contain beta-carotene pigments and with respect to genotypes, developmental stages, and environmental factors, the beta-carotene pigment level varies which is responsible for yellow and orange colour in fruits. According to Alquizar *et al.* (2018) and Giménez-Sanchis *et al*. (2022) the pulp's red colour was caused by a high lycopene content or a high anthocyanin content, while its white colour was caused by a high lutein content. Carotenoids have health-promoting properties, and a higher intake of carotenoids is reported to reduce the risk of cancer (Colditz, 1987). This nutritional advantage could be attractive from a marketing standpoint.

5.1.16 Pulp colour intensity

The colour intensity of the pulp is an important characteristic that influences the appeal of the fruit to consumers (Tadeo *et al*., 2020). Pulp colour intensity was perceived as dark for six genotypes whereas light intensity of pulp colour was recorded in four genotypes. The findings are similar to the report by Gaikwad *et al.* (2015) who found light and dark shades of pulp colour in different pummelo genotypes. Pulp colour intensity depends on the accumulation of colour-inducing pigments such as beta-carotene and anthocyanin (Xie *et al*., 2023). The synthesis, degradation and retention pathways of fruit pigments are mediated by hormonal, genetic, and environmental factors (Kapoor *et al.*, 2022). Fruits grown in warm summers followed by cold winters tend to have more intense pigmentation (Cebadera *et al*., 2019).

5.1.17 Seeds per fruit

The presence of a large number of seeds in *Citrus* fruits can be a significant obstacle to consumer acceptability, regardless of the fruit's excellent taste and texture (Raza *et al*., 2003). Due to their seediness, a number of cultivars with desirable horticultural traits have not gained commercial importance (Fatta-Del-Bosco *et al*., 1992). On the other hand, a large number of seeds is a desirable quality in breeding programmes (George *et al.*, 1999). The fact that most seedless fruits are small suggests that some kind of stimulation, maybe hormonal, from the seeds (ovules) controlling fruit growth (Ladaniya, 2008). The average number of seed count was 21.73 and the range varied from 8.66 to 47.67. Similar findings were also quantified by Singh *et al.* (2009) who reported that the average number of seeds

among 28 lemon genotypes was 20.64, and by Sharma *et al*. (2015) who found a range of 1.00 to 48.33 seeds per fruit in grapefruit cultivars. Rabha *et al.* (2013) also reported a range of 5 to 48 seeds per fruit in 32 *Citrus* genotypes from Arunachal Pradesh. There may be genetic differences that lead to the variation in seed number per fruit between species (Dorji *et al*., 2011; Marboh *et al*., 2015). High levels of inter- and intra-species cross-pollination may also be a contributing factor to the variation. (Kahn and Vidalakis, 2014).

5.1.18 Seed shape

The shape of a seed is a crucial characteristic when it comes to identifying and classifying plants. Seed shape is also significant as it is indicative of genetic, physiological, and ecological factors that influence quality and market value (Cervantes *et al*., 2016). Variation in seed shape was observed as four genotypes showed an ovoid seed shape, two genotypes showed a clavate seed shape, two genotypes showed a semi-deltoid seed shape and one genotype showed a cuneiform seed shape. The findings are in accordance with the study carried out by Jaskani *et al*. (2006) and Gaikwad *et al.* (2018), who reported wide variation in seed shapes such as semi-deltoid and ovoid in rough lemon genotypes, clavate and spheroid in bittersweet orange, and clavate and cuneiform in sour orange. The seed shape varies greatly among species (Ladaniya, 2008). The variation in seed shape is due to genetic factors (Dorji *et al*., 2011). According to a study by Altaf *et al.* (2008) on Kinnow mandarin, variations in seed shape may also result from the crossing of plants with various pollen sources.

5.1.19 Seed colour

Seed germination and dormancy have been reported to be influenced by seed colour (Powell, 1989). Many studies have demonstrated that seed colour influences water uptake (Powell, 1986). Cream seed colour was observed in most of the genotypes while white and creamy white seed colour occurred in one genotype each. Variation in seed colour was also observed by Gaikwad *et al.* (2018) in rough lemon genotypes as yellow, cream and brown, and by Singh *et al.* (2010) in rangpur lime genotypes as brown and cream. Jaskani *et al.* (2006) documented white seed colour in sour orange and bitter-sweet orange and Malik *et al.* (2006) documented cream seed colour in *Citrus indica*. The differences in seed colour are a result of genotypic differences and the influence of the environment. (Singh *et al*., 2010).

5.2 Biochemical Characteristics

5.2.1 Moisture content

Fruits serve as a reservoir for moisture, and when leaves lack moisture, they drain moisture from fruits (Ladaniya, 2008). The moisture level in fruit directly impacts its shelf life and the potential for microorganisms to thrive and reproduce (Nasir *et al.*, 2004). The rind is more leathery when it has less moisture (Ladaniya, 2008). A high moisture content of more than 70% was observed in the peel and pulp of all the *Citrus* cultivars. The moisture content in peel was found to be averaging at 75.59% and that of pulp to be at 88.46%. The findings of the present study were found to be in line with the findings by Tripodo *et al.* (2004) who found a moisture content of 85.7% and 88.6% in pulp of orange and lemon. Supporting results were also reported by Gaikwad *et al.* (2015) who found that the moisture content in Pummelo genotypes ranged from 82.1% to 91.05%. The moisture content of the pulp was greater than that of the peel owing to the juice content in the pulp. A high moisture content of the pulp is to be expected because *Citrus* fruits are known for being juicy and are mostly taken to quench the taste (Ani and Abel, 2018). Low levels of moisture of 23.75% in rough lemon and 16.20% in *Citrus maxima* peels were also documented. (Mohammed *et al.*, 2013; Ani and Abel., 2018). Usually, this variation is a result of different cultivars, geographic locations, techniques of drying and well as the stage of harvesting (Tadeo *et al.*, 2008).

5.2.2 Juice pH

A high pH level of *Citrus* fruits is an attribute that contributes to their immense popularity as fresh fruit. (Herath *et al*., 2016). The pH content for the present study was found to be averaging at 3.32 and had a range of 2.60 to 3.90. The pH level of all the cultivars was found to be much less than 7 which meant that fruits were highly acidic. A similar finding was also reported by Shravan (2018) who found that the pH of *Citrus sinensis* fruit juice was 3.8. Similar observations have also been recorded by Herath *et al.* (2016) as they observed a pH of 2.37 in lime, 2.54 in lemon, 2.88 in sour orange, 3.42 in grapefruit and 3.98 in pummelo. As pH decreases, the acids become more undissociated and impart more of a sour taste (Dziezak, 2016). Juice pH variations arise from variations in acid content between genotypes, which are influenced by the genetics of the individual plant and environmental factors (Marboh *et al*., 2015).

5.2.3 Total soluble solids (TSS)

TSS is an important indicator of the quality of fruit and its flavour. The Brix percentage is crucial for the industrial processing of *Citrus* fruits into commercial beverages and jams (Tadeo *et al*., 2008). The TSS is contributed by sugars, acids, dissolved vitamins, proteins,

phenolic compounds, minerals and pigments. Of these components, sugars make up approximately 75-85% of the total soluble solids (Ncama *et al*., 2017). A higher TSS/ total acid ratio is directly correlated to sweetness in the fruit (Ranganna, 1986). A great variation in TSS of the different *Citrus* cultivars was observed in the present study with TSS ranging from 5.30 to 11.40 ℃Brix. The findings were comparable with those obtained by Singh (2004) who found a TSS range of 7.08 to 10 ℃Brix in Pummelo genotypes. A similar result was obtained by Josan and Kaur (2006) who studied 25 Mandarin types and found their TSS to vary from 6.13 to 12.20 ℃Brix. According to Dorji and Yapwattanaphun (2011), the influence of elevation and climatic conditions on variance in TSS could not be ignored. The variation in TSS is attributed to sugar and acid content that arises due to genetic differences, the age of the tree, the stage of harvesting and environmental factors (Frometa and Echazabal 1988; Ladaniya, 2008; Erkan and Dogan, 2019). Fruit position on the tree also influences TSS as fruit near the top and edges of the canopy are more exposed to sun radiation and may have higher levels of total soluble solids (Tadeo *et al*., 2008). Heavy vegetative growth flushes will deplete the resources that the fruit is able to use to develop, which will lead to fruits with decreased TSS (Zekri *et al*., 2011).

5.2.4 Titratable acidity

The attribute of acidity is significant in determining the acceptability of *Citrus* juice as tartness plays a major role. This is because acid is responsible for imparting the characteristic sour taste to the product (Ahmad *et al*., 2008). The acidity of the juice is also a factor in determining its suitability for processing (Voragen *et al*., 1991). The titratable acid in a fruit juice is contributed mainly by citric, malic, acetic and tartaric acids (Tyl and Sadler, 2017). The titratable acidity percentage ranged from 0.97% to 4.71% with a mean value of 3.13%. Comparable results were obtained by Sayed *et al.* (2006) who reported a mean titratable acidity of 4.50% in rough lemon and by Singh *et al.* (2010) who reported mean titratable acidity of 3.4% to 5% in rangpur lime. Low titratable acidity was obtained by Hangsing *et al.* (2016) and Kakoti *et al.* (2019) in Khasi mandarin (0.73-0.81% and 0.65% respectively). A high percentage of titratable acidity was reported by Gaikwad *et al.* (2018) which ranged from 3.53% to 8.72% in Rangpur lime genotypes and 3.49% to 7.47% in rough lemon. Variations in titratable acidity are a result of varietal differences, the effect of climate and soil, the age of the tree and the stage of harvest (Frometa and Echazabal, 1988; Ladaniya, 2008).

5.2.5 Ash content

Ash is the term used to describe the inorganic residue left behind either after complete oxidation or ignition of organic materials in a food product (Marshall, 2010). Ash content represents the total mineral content in food (Marshall, 2010). Ash content can be significant from a dietary, toxicological, and food quality perspective since some foods are abundant in specific minerals (Harris, 2017). The first step in preparing a food sample for elemental analysis is ashing. In the current study, the ash content was found to be averaging at 4.26% for pulp and 4.02% for peel with a range of 2.83% to 5.96% and 2.53% to 5.69% respectively. The present findings are in line with that of Mathias *et al.* (2019) who found that ash content in *Citrus* peel to be 5.57% and that of Suri *et al.* (2022) who concluded that sweet lime peels had an ash content of 5.93%. Results reported by Joseph (2016) in *Citrus* sinensis peels showed an ash content of 14.76% which is not comparable to the results in the present study. The variation in ash content is a result of genetic factors, soil management practices, geographic location or stage of harvest (Ladainiya *et al*., 2008).

5.2.6 Crude protein

Nitrogen-containing compounds play a crucial role in plants as they are found in various structural and productive organs including fruits, and are important for metabolism (Ladaniya, 2008). The crude protein is an attribute where the total nitrogen content is used to determine the amount of protein in the food (Henneberg, 1865). The crude protein level is a significant factor in determining the nutritional value of the fruit. The protein is also required to maintain continuous evolution of ethylene which leads to ripening (Hyodo, 1981).

The protein content in pulp had a mean value of 7.45% with a range of 5.6% - 9.16% and that in peel had a mean value of 8.744% with a range of 5.6% to 13.83%. The findings of the present study were in consonance with the reports of Tripodo (2004) who found the protein content in orange and lemon pulp to be 8.6% and 7.6% respectively. Similar findings were also reported by Olabinjo *et al.* (2017) who found the protein content in sweet Orange peel to be 7.15% and by Suri *et al.* (2018) who found 8.18% protein in peels of sweet Lime. The high protein content in *Citrus* makes it a nutritious food. A significant protein content in peel also makes it an excellent feed for animals. A low crude protein concentration (4.2mg/100g) was found in *Citrus maxima* peel (Ani and Abel, 2018). The variation in crude protein content is a result of genetic factors, soil and climatic conditions, maturity of the fruit, drying method used or storage conditions (Pascual and Carmona, 1980; Ladaniya, 2008).

5.2.7 Reducing Sugars

As a fruit reaches its mature stage, carbohydrate polymers break down and starch turns into sugar, affecting the fruit's flavour and texture, making it sweeter and softer (Ladaniya, 2008). The higher rate of browning in fruit can be cited as the abundance of sugars, particularly reducing sugars (Raju and Bawa, 2012). Reducing sugars are easily absorbed by the body because they don't need to be broken down and thus, they can serve as instant source of energy (BeMiller, 2017). The reducing sugars in the juice of the different germplasms under study ranged from 2.06% to 6.72% and had an average value of 3.97%. The present findings of this study are consistent with the results by Diwan *et al.* (2014) who found an average reducing sugar content of 4.08% in 28 Sweet orange varieties. Moreover, Gaikwad *et al.* (2018) and Kumar *et al.* (2011) also found comparable values for reducing sugar which ranged from 1.68% to 2.63% in Rangpur lime genotypes, 1.46% to 2.82% in rough lemon genotypes and 4.41% to 6.52% in Mandarin genotypes. The reducing sugar percentage was higher than the non-reducing sugar percentage in all the cultivars. This is because the non-reducing sugars of juice such as sucrose are converted into reducing sugars during ripening (Pruthi *et al.*, 1984). The variation in reducing sugar content across the cultivars arises due to genetic differences, maturity stage, varying soil and climate and stage of harvesting (Ting *et al.*, 1971; Marsh *et al.*, 2000)

5.2.8 Non-reducing sugars

Sucrose is the major non-reducing sugar in *Citrus* fruits (Matheyambath, 2016). The non-reducing sugar content in the juice of the cultivars under study varied from 0.48% to 4.15% and had an average value of 2.54%. Similar results were obtained by Gaikwad *et al.* (2018) as they found non-reducing sugar to range from 0.88% to 3.17% in Rangpur lime and 1.63% to 3.93% in rough lemon. The results are also in line with the findings of Diwan *et al.* (2014) who found a mean non-reducing sugar of 2.62% in Sweet orange varieties. The variations between species result due to the genetic makeup of the accessions, geographical conditions and harvesting stage (Ting *et al.*, 1971; Marsh *et al.*, 2000).

5.2.9 Total sugar

The total sugar includes both reducing and non-reducing sugars. An increase in total sugar indicates advancement through maturation and ripening (Liu *et al*., 2012). Sugars play a crucial role as essential nutrient factors and can also act as signals that trigger specific hormonal responses. (Goldschmidt and Koch, 1996; Iglesias *et al*., 2006). The sweetness of *Citrus* juice is related to its total sugar content (Zhang *et al.*, 2016). The total sugar content

in the *Citrus* species ranged from 2.54% to 9.36% and the mean total sugar percentage across all the cultivars was 6.52%. The results are in close conformity with the findings of Rabha *et al*. (2013) who found a total sugar range of 0.20% to 10.21% among different *Citrus* genotypes of Arunachal Pradesh. Results reported by Hazarika *et al.* (2012) in pummelo showed total sugar content of 7.47%- 9.95% which is comparable to the present findings.

5.2.10 Organic acids

Organic acids are among the principal compounds in fruits producing a sour sensation or acidity. Fruit acidity is also one of the essential factors to decide the harvest time in crops where acidity is important for consumers' acceptance (Baldwin, 2002). The organic acid content of fruits is intriguing since it has a significant impact on the sensory qualities of fruits and fruit juices (Karadeniz, 2004). Each fruit has a unique pattern of organic acids (Wrolstad, 1981). In addition, several organic acids may be utilised as indicators of ripeness, bacterial activity, and adulteration (Palmer and List, 1973; Evans *et al.*, 1983; Blanco *et al*., 1996).

Seven organic acids were quantified out of which Citric acid was the most predominant acid. Citric acid is the first intermediate of TCA cycle. Citric acid as the dominant acid in *Citrus* juice was also confirmed by Clements (1964) and Yamaki (1989). The Citric acid content ranged from 18.41 mg/100 ml to 1807.89 mg/100 ml and had a mean value of 720.5 mg/100 ml. The results are comparable with the findings by Duarte *et al.* (2010) and Kardeniz (2004) who found a citric acid content of 34 to 966 mg/100 ml in mandarins, 633 to 1288 mg/100 ml in oranges and 1110 to 1565 mg/100 ml in sweet orange. It was followed by malic acid having an average value of 313.15 mg/100 ml and a range of 16.98 mg/100 ml to 623.64 mg/100 ml. Malic acid also occurs as an intermediate of TCA cycle. Similar findings were reported by Violeta *et al.* (2010) who found malic acid values of 87.1 mg/100 ml in pomelo, 177 mg/100 ml in mandarin and 518.3 mg/100 ml in lime. Ascorbic acid which is an important antioxidant was found in a range of 9.66 mg/100 ml to 297.09 mg/100 ml in the juice of the *Citrus* cultivars. The amount of ascorbic acid was comparable to the findings of Violeta *et al.* (2010) and Duarte *et al.* (2012) who reported an ascorbic acid content of 34 to 56.4 mg/100 ml in mandarins, 33.8 to 86 mg/100 ml in oranges and 35 mg/100 ml in lime. Ascorbic acid acts as immunity booster and lowers blood cholesterol level in the body (Iqbal *et al.*, 2004). The anti-scurvy property of ascorbic acid has been well documented by Hodges *et al.* (1969). The Smirnoff–Wheeler pathway, in which vitamin C is synthesized from d-mannose and l-galactose (d-mannose/l-galactose

pathway) represents the major route of Vitamin C biosynthesis in the plant (Smirnhoff, 2018). However, it should be highlighted that Sweet orange juice has a significantly higher concentration of this acid than the other fruits. It could be said that 50 ml of fresh Sweet orange juice would be sufficient to provide the daily RDA for ascorbic acid (RDA for ascorbic acid is 40-80 mg/day as per ICMR, 2010).

Tartaric acid, Fumaric acid and Succinic acid are powerful immunity boosters and antioxidants are present in *Citrus* juices in variable amounts. The value of tartaric acid ranged from 46.27 to 171.81 mg/100 ml. Fumaric acid and Succinic acid content ranged from 1.71 to 118.78 mg/100 ml and 57.97 to 637.65 mg/100 ml respectively. The present findings are not in conformity with the results of Chinnici *et al.* (2005), Violeta *et al.* (2010) and Kelebek *et al.* (2010) as they found a low concentration of the above acids in the *Citrus* juice. The presence of oxalic acid in the fruit juice is harmless in small amounts but higher amounts may contribute to kidney stone formation and reduce the mineral absorption of the body. Oxalic acid was present in low concentrations in the range of 7.53 to 30.83 mg/100 ml in the *Citrus* juice. These results were comparable to the findings of Duarte *et al.* (2012) and Violeta *et al.* (2010) who reported oxalic acid content of 4.9 mg/100 ml in clementine, 8.8 mg/100 ml in Mandarina and 26.8 mg/100 ml in pomelo.

5.2.11 Water-soluble vitamins

Vitamins are organic substances that are necessary for appropriate physiological functioning. Since most vitamins cannot be produced by the body on their own, they must be consumed in sufficient quantities through diet (Kennedy, 2016). Five water-soluble vitamins were quantified namely thiamine, niacin, pyridoxine, pantothenic acid and folic acid. These vitamins were found in most fruits in varying amounts. Vitamin B_1 or thiamine was present in the highest amounts among all the B group vitamins in all fruits. The average concentration was 59.00 mg/100 g and the range was 7.77 to 125.71 mg/100 ml. These results were also supported by Okwu (2008) who reported the thiamine concentration to be the highest in various *Citrus* cultivars such as *Citrus reticulata* and *Citrus aurantifolia*.

Niacin and pyridoxine were identified in concentrations ranging from 5.18 to 26.26 mg/100 g and 3.30 to 79.78 mg/100 g respectively. The results are comparable with the findings of Ani and Abel (2017) who found niacin and pyridoxine content of 14.42 mg/100 ml and 10.51 mg/100 ml in *Citrus maxima* juice. Pantothenic acid content was found in the range of 1.77 to 21.83 mg/100 g in the fruit juices which is higher than the content reported by Ladaniya (2008) in grapefruit, valencia orange, navel orange and eureka lemon, and

Trong *et al.* (2018) in lemon. The folic acid concentration ranged from 0.11 to 0.52 mg/100 ml across the cultivars which could be supported by the findings of Ani and Abel (2017) and Saeid and Ahmed (2021) who found a folic acid concentration of 0.60 mg/100 ml and 0.11 mg/100 ml respectively in *Citrus maxima* and *Citrus limon* cultivars. It could be concluded that 100 ml of fresh Sweet orange juice would be sufficient to provide the daily RDA for folic acid (RDA for folic acid is 0.4 mg/100 g (ICMR, 2010)). The variation in the water-soluble vitamins among the cultivars is due to genotypic differences, climatic and soil conditions and cultural practices (Lee and Kader, 2000). The vitamin content of fruits can also be affected by factors such as how they are harvested, transported, and stored. (Pertuzatti *et al*., 2015).

5.2.12 Mineral analysis

The physiology and metabolism of *Citrus* plants and fruits depend heavily on mineral components. A large number of these chemicals are essential components of human nutrition (Ladaniya, 2008). The major and minor minerals in the pulp and peel of *Citrus* genotypes varied significantly among the genotypes. It could be observed that the mineral contents of the peels were significantly higher than those of the pulps**.** This was also evident in the findings by Czech *et al.* (2020) who also found that the fruits' peels contained significantly more macro and micronutrients than their pulp. The *Citrus* species are good a source of major minerals such as sodium, magnesium, potassium and calcium. Sodium plays a crucial role in maintaining the balance of water and electrolytes in the body (Pohl *et al.,* 2013). The average sodium content in peels was 45.14 mg/100 g. The findings are comparable to the findings of Barros *et al.* (2012) who found a sodium content of 32.4 mg/100 g in Ponkan mandarin, 37.7 mg/100 g in sweet lime, 54.0 mg/100 g in Tahiti lime and 85.10 mg/100 g in Lima orange. The average sodium content in the pulp was 14.33 mg/100 g which is higher than the results reported by Barros *et al.* (2012) and Paul *et al.* (2004) in *Citrus species*. The average magnesium content in the peels was 178 mg/100 g and that in the pulp was 69.83 mg/100 g. The magnesium content of the cultivars under study was higher than the magnesium content that was documented by Paul *et al.* (2004) in *Citrus* pulp and Barros *et al.* (2012) in *Citrus* peel and pulp under their study.

The potassium content of the genotypes under study averaged at 21.20 mg/100 g in the peels and 10.46 mg/100 g in the pulp. Higher potassium content was documented by Paul *et al.* (2004) and Barros *et al.* (2012) in *Citrus* species under their study. *Citrus* fruits are a valuable source of calcium, which plays an important role in building hard, strong bones

(Baghurst *et al.*, 2003). A high calcium content was found in all the species that averaged at 150.75 mg/100 g for peels and 38.53 mg/100 g for pulp. This was in agreement with the findings of Czech *et al.* (2020) who reported a calcium content in the pulp as 41.9 mg/100 g in Orange, 28.8 mg/100 g in Pomelo, 37.1 mg/100 g in Mandarin, 31.8 mg/100 g in Lemon, 63.9 mg/100 g in Key lime and 36.0 mg/100 g in red grapefruit. The analysis showed that the pulp of *Citrus* fruits provides approximately 4% (in a mandarin weighing about 100 g) to 31.5% (in a pummelo weighing about 600 g) of the RDA for calcium (RDI for Calcium is 800 mg per day (ICMR, 2010)).

Iron was found to be the dominant micronutrient present in both the peel and pulp of the *Citrus* fruits that were analysed. This was in accordance with the findings by Czech *et al.* (2020) who also found iron to be the primary micronutrient among all the species under study. The average iron content was 13.09 mg/100 g in the peel and 10.25 mg/100 g in the pulp. This was in accordance with the results of El-Beltagi *et al.* (2022) who found an iron content of 16 mg/100 g in *Citrus sinensis* pulp but not in accordance with the findings of Barros *et al.* (2012) who found a lower content. However, it should be highlighted that Sweet orange pulp has a significantly higher concentration of this mineral than the other edible fruits. The rich content of iron in *Citrus* fruits is important information, especially for those with high iron needs, such as pregnant women and women of childbearing age (for whom the pulp from one mandarin of 100 grams would provide approximately 33% of the RDI) (RDI for Iron is 17 mg per day (ICMR, 2010)). The copper content was found to have an average value of 0.83 mg/100 g in the peel and 0.31 mg/100 g in the pulp. These results were comparable to the findings of Paul *et al.* (2004) who found copper content of 0.26 mg/100 g in Lemon and 0.20 mg/100 g in orange. The addition of *Citrus* to the di*et al*so provides a sufficient quantity of copper to the body and fulfils the RDI of 1.2 mg per day up to a great extent (ICMR, 2010).

Another vital micronutrient is zinc, which strengthens the immune system and guards the body against oxidative stress (Chasapis *et al.*, 2012). The average zinc content that was observed was 3.37 mg/100 g in peel and 1.28 mg/100 g in pulp. The present findings are in conformity with the findings of Singh *et al.* (2015) in grapefruit. The analysis showed *Citrus* fruit pulp would provide between 3.2% (in a mandarin weighing about 100 g) to 21.6% (in a pummelo weighing about 600 g) of the RDI of zinc (RDI of zinc is 15 mg per day according to ICMR, 2010). Heavy metals such as cadmium and lead have been identified in very trace amounts in both the peel and pulp of *Citrus* species. The average cadmium content in the peel and the pulp was 0.004 and 0.003 respectively which is much lower than the permissible

limit for cadmium in the diet recommended by WHO (i.e., 0.02 mg/100g). Similarly, the average lead content in the peel and the pulp was 0.003 and 0.001 respectively which is much lower than the permissible limit for lead in the diet recommended by WHO (i.e., 0.03 mg/100g). Fruit's mineral content may be affected by the mineral content of the soil in which it was produced, the mineral content of irrigation water, weather conditions, and agricultural practices, such as the types and quantities of fertilizers incorporated (Czech, 2019).

5.2.13 Essential oil content

Essential oils extracted from *Citrus* peel are an important by-product of the *Citrus* industry (Lu *et al.*, 2019). The essential oil yield of *Citrus* peels ranged from 0.26% to 0.83% with an average yield of 0.48%. The results are in line with the findings by Javed *et al* (2014) who documented an essential oil yield of 0.45%, 0.37% and 0.23% in *Citrus paradisi, Citrus sinensis and Citrus reticulata* respectively. According to previous works of Lota *et al.* (2000), the essential oil yield in cultivars of mandarin ranged from 0.1% to 0.45%. Similar results were also obtained by Kamal *et al*. (2013) who found essential oil yields of 0.30%, 0.20% and 0.24% in *Citrus reticulata, Citrus paradisi and Citrus sinensis* respectively. According to Tu *et al.* (2002), the output of *Citrus* essential oils varied with each plant species, typically ranging from 0.2-2.0%. Tao *et al.* (2009) and Hamdan *et al.* (2010) reported an essential oil yield of 2.49% in Bingtang sweet orange and 4% in *Citrus jambhiri* respectively which is higher than the present findings. Essential oil yields vary depending on the genotype of the plant, the harvesting stage, the extraction process, the extraction unit, the soil, and the climate (Huet, 1991). According to Bhuyan *et al.* (2015), the oil content of Khasi mandarin fruit peel increased in the early stages of fruit development up until the turning stage and then began to decline as the fruit matured.

5.2.14 Major volatile compounds in essential oil

Essential oils synthesized in the plant body bring about numerous biological activities such as to allelopathy, adaptation to abiotic stresses and intra and inter plant signalling (Sharifi-rad *et al.*, 2017). Direct defense responses of the plant comprise plant volatiles that are toxic and repellent; in addition, they can be antinutritional agents and reduce digestibility, growth and reproduction against natural enemies (War *et al.*, 2011). The analysis of volatile compounds in essential oil revealed 8-13 major volatile compounds across the *Citrus* species. D-limonene was the dominant volatile compound in the essential oil and was present in the range of 45.7% to 81.58%. D-limonene as the chief chemical constituent in the essential oil of *Citrus* peel was also documented by Svoboda & Greenaway (2003), Javed *et*

al. (2013), Zhang *et al.* (2022) and Merle *et al.* (2004). They reported the limonene content in *Citrus* peels to range from 32%-98% in various cultivars. D-limonene is also the principal odour component in the *Citrus* species and is responsible for the characteristic lemon-like aroma of the fruits (Minh Tu *et al.*, 2003). The biological functions of D-limonene are immense due to its antibacterial, antifungal, and anti-tumoral properties (Graebin *et al.*, 2010). High D-limonene content is also an indicator of good radical scavenging activity (Roberto *et al.*, 2010).

Other major compounds in the essential oil of *Citrus* peels were γ-terpinene, α-pinene, β-pinene and linalool. These results were supported by the findings of various scientists such as Lota *et al.* (2000), Amorim *et al*. (2016), Malhotra *et al*. (2008), Kamal *et al*. (2013) and Bousbia *et al.* (2009). Other scientists such as Jirovetz *et al.* (2005) and Bajpai *et al.* (2007) have reported principle active compounds such as citral, eugenol, thymol, and cineol in *Citrus* essential oil which is not in conformity with the present findings. γ-terpinene is an antioxidant and anti-microbial (Li *et al.*, 2009) but it contributes to flavour deterioration because it is degraded to *p*-cymene (Ikeda *et al*., 1962). The antifungal and antimicrobial properties of α-pinene and ß-pinene have been well established which protects the plant from biotic stresses (Bora *et al*., 2020). Linalool is the major alcohol responsible for the sweet and floral smell of the *Citrus* peel essential oils (Kostadinovic *et al.*, 2014) and possesses anti-microbial activity (Javed *et al*., 2014). Linalool is one of the compounds responsible for the flavour of *Citrus* fruits. (Fang *et al.*, 2004) The presence of such compounds in *Citrus* essential oil makes it ideal for use in food and health industry, perfumery industry and cosmetic industry.

The uniqueness in flavour and aroma of the *Citrus* cultivars can be perhaps attributed to the unique type of compounds that are present in certain cultivars and not present in others. Camphene has been identified in Rangpur lime which might be perhaps responsible for the unique aroma of the fruit which is different from other cultivars. Camphene has been proven to be useful for pain relief, has antioxidant, anti-fungal and anti-viral effects and the Rangpur lime peels can serve as a biological source of camphene. The strong scent of Bira jora fruits can be perhaps attributed to the presence of a high percentage of α -terpineol (28.06%) and the presence of unique compounds like cis-α -bergamotene, ß-bisabolene and 3-carene in the peel and the interaction between them. Myrcene and nerol are both characterized by floral, citrsy, and balsamic odour (Lan Phi *et al.*, 2006) and are present exclusively in Sweet orange which might be responsible for their fresh and characteristic scent. Thymol is an important flavour compound in sweet orange and acts as an essential antibacterial component in the

fruit (Shaw, 1979). Sweet orange can also serve as a biological source of myrcene and nerol which are used as an intermediate in the fragrance and flavour industry and of thymol which is used in pesticides and disinfectants. The mild and grassy flavour of Khasi papeda fruits might be due to the presence of geranial which is absent in other cultivars. Geranial is one of the most important compounds in the fragrance industry and is seen to exhibit insecticidal and insect-repellant properties (Chen *et al*., 2010). Khasi papeda fruits can thus serve as a biological source of geranial for industries as it has a high percentage of geranial (0.71%). Eucalyptol is an ingredient in mouthwashes, cough medications, and insect repellents and has been identified exclusively in Kaffir lime essential oil. Eucalyptol in high concentrations is toxic and is found to cause headaches, nausea, and vomiting and therefore should be used with care. *Citrus* essential oils can also be employed as natural preservatives to extend the shelf life of food due to their anti-fungal and anti-bacterial nature. The ecological function of the presence of essential oil in the peels is that it protects the fruits from the attack of pests and diseases as they have anti-microbial properties. In fruits such as Bira Jora which bear close to the ground, the presence of essential oils protects it from soil microbes as well. The essential oil also enhances the shelf life of the fruits after harvest due to their preservative properties. The composition of essential oils can vary depending of various factors such as variety, geographical region, season, age of the plant and the essential oil drying and extraction method.

Summary and Conclusion…

CHAPTER VI

SUMMARY AND CONCLUSION

The present investigation was conducted to characterize some of the *Citrus* germplasm of Assam. Ten accessions representing the five groups of *Citrus* were procured from AAU Citrus and Plantation Crop Research Station, Tinsukia and CCRI (ICAR), BNCA Campus, Biswanath Chariali, Assam. The cultivars studied were Rangpur lime, Bira jora, Sour orange, Sweet orange, Khasi mandarin, Cleopatra mandarin, Pummelo, Grapefruit, Kaffir lime and Khasi papeda. In this chapter, the major findings of the present study are summarized and concluded below:

1. Studies on morphological fruit quality characteristics revealed considerable variation among the genotypes in terms of fruit shape (spheroid and ellipsoid), shape of fruit base (convex, concave collard, concave, truncate, necked), shape of fruit apex (rounded, mammiform, truncate) fruit skin (epicarp) colour (orange-green, yellow, orange, yellow-green, dark yellow,) fruit surface texture (pitted, bumpy, rough, papillate, smooth), albedo colour (white and pink) adherence of albedo (strong and weak), fruit axis (semi-hollow, solid, hollow), pulp colour (orange, white, yellow, pink), pulp colour intensity (dark and light), seed shape (ovoid, clavate, semi deltoid, cuneiform) and seed colour (creamy, white, creamy white).
2. Considerable variations among the genotypes in terms of morphological quantitative characters were observed such as fruit weight (50.37 to 826.40g) fruit length (5.77 to 14.57 cm), fruit breadth (5.57 to 15.28 cm), number of segments (9.67 to 14.33), fruit rind thickness (1.23 to 16.10 mm), juice content (14.95 to 50.84%) and seeds per fruit (8.67 to 47.67).
3. Significant variation was observed among the *Citrus* cultivars with respect to the biochemical characters studied.
4. Moisture content varied from 84.30% to 92.51% in the pulp and 70.20% to 83.32% in the peel and the highest moisture content was recorded in Rangpur lime pulp and peel respectively.

5. Juice pH varied from 2.60 to 3.90 and the highest pH was recorded in Khasi mandarin.
6. TSS of juice ranged from 5.30 Brix to 11.40 Brix and was highest in Sweet orange.
7. Titratable acidity of the juice varied from 0.97% to 4.71% and the highest value was recorded in Kaffir lime.
8. Ash content varied from 2.83% to 5.96% in the peel and 2.53% to 5.69% in the pulp and the highest value was recorded in Rangpur lime peel and Sour orange pulp respectively.
9. Reducing, non-reducing and total sugar ranged from 2.06% to 6.72%, 0.48% to 4.15% and 2.54% to 9.36% respectively and the highest values were recorded for Khasi mandarin, Sweet orange and Khasi mandarin respectively.
10. Crude protein content varied from 5.60% to 9.16% in the pulp and 5.60% to 13.83% in the peel and the highest crude protein contents were recorded for Sour orange pulp and Bira jora peel respectively.
11. Organic acid profile was determined using UHPLC and seven organic acids were quantified. Citric acid was found to be the most dominant among all. All the *Citrus* species were found to be a good source of ascorbic acid.
12. Five water-soluble vitamins were quantified using UHPLC namely thiamine, niacin, pyridoxine, pantothenic acid and folic acid out of which thiamine was present in the maximum amount in all the cultivars
13. Mineral content in the peel and the pulp was detected with the help of ICP-MS. The decreasing trend of minerals was Mg >Ca >Na >K >Fe >Zn> Cu in the peel and the pulp. Heavy metals such as cadmium and lead were present in trace amounts
14. Essential oil content (%) varied from 0.26% to 0.83% and maximum yield was recorded in Rangpur lime.
15. Analysis of volatile compounds in the essential oil was carried out with the help of TQGCMS/MS which revealed the presence of D-limonene as the principal compound and other major compounds such as α-pinene, ß-pinene, γ -terpinene, α-terpineol, linalool and others in varying quantities.

CONCLUSION

The biochemical study of the genotypes is an essential part of their characterization which has been carried on for the first time in the given *Citrus* fruits. The present study indicates that there is a presence of significant variability among the ten genotypes of *Citrus* which grow naturally in wild and semi-wild conditions in terms of their morphological and

biochemical characteristics. The analysis of the biochemical composition of the fruits shows that they exhibit excellent nutritional value as they are a good source of protein, sugar, vitamins and minerals and should be incorporated into our daily dietary plan for various individual groups. The organic acid profile shows citric acid and ascorbic acid in abundance which makes them a good source of antioxidants for the body. Since the requirements of Ascorbic acid (vitamin C) is the highest in human, these findings facilitate the incorporation of citrus fruits having relatively higher Ascorbic acid in the dietary plan. The peels are also an important part of the fruit which is generally treated as waste but can be utilized as they are rich in phytonutrients. The high percentage of essential oil in peels and the presence of important volatile compounds facilitate the selection of citrus genotypes ideal to be utilized for specific domestic and industrial purposes. Furthermore, the findings are expected to facilitate the commercial exploitation of the citrus germplasm from this region including those of underutilized crops for their nutritional, pharmaceuticals/ nutraceuticals and other industrial importance.

FUTURE PROSPECTS

On the basis of the investigation, the following suggestions are made for future study:

- Conservation of the genetic diversity and improvements of *Citrus* using appropriate methods and utilization of the species for their wider applications.
- Complete qualitative / Quantitative Phytochemical profiling of all the citrus germplasm from this region in search of nutritional, pharmaceuticals/ nutraceuticals other industrial importance for their proper and commercial plantation and utilizations.
- Studies on the preparation of value-added products, enrich diet formulation, industrially significant nutraceuticals and pharmaceuticals compounds is an important area to be explored.
- Valorization of *Citrus* peel as functional food.

Bibliography…

BIBLIOGRAPHY

Abad, M.K.R. and Besheli, B.A. (2016). Insecticidal potential of essential oil from the leaves of *Citrus aurantium* L. against *Oryzaephilus surinamensis* (F.), *Lasioderma serricorne* (L.) and *Sitophilus oryzae* (L.). *J. Entomol. Zool. Stud.*, **4**: 865–869.

Agriculture Statistics at a Glance (2022). Government of India, Directorate of Economics and Statistics, Department of Agriculture, Cooperation and Farmers Welfare. https://agricoop.gov.in/en/Agricultural_Statistics_at_a_Glance.html retrieved on 28 March 2023

Ahmed, M.; Ahmad, A.; Chatha, Z. A. and Dilshad, S. M. R. (2008). Studies on preparation of ready to serve mandarin (*Citrus reticulata*) diet drink. *Pak. J. Agri. Sci.*, **45**(4): 470-476.

Ahuja, A. (1996). Loss of biodiversity and need for conservation. In: *Changing Perspectives of Biodiversity Status in the Himalaya*, Gujral, G.S. and Sharma, V. (Eds.). The British Council, New Delhi, India.

Ajon, G. (1943). The pigments of Citrus juice during ripening. *Riv. Ital. EPPOS*, **25**: 150-157.

Akhter, S.; Ferdous, M. J.; Hossain, M. R. and Rabbani, G. (2009). Molecular characterization of Jamir (*Citrus jambhiri*) accessions of Bangladesh through PCR based RAPD markers. *Journal of Agrofor. Environ.*, **3**(1): 21-24.

Akiyama, S.; Katsumata, S.; Suzuki, K.; Ishimi, Y.; Wu, J. and Uehara, M. (2010). Dietary hesperidin exerts hypoglycemic and hypolipidemic effects in streptozotocin-induced marginal type 1 diabetic rats. *J. Clin. Biochem. Nutr.*, **46**(1): 87-92.

Akpata, M. I. and Akubor, P. I. (1999). Chemical composition and selected functional properties of sweet orange (*Citrus sinensis*) seed flour. *Plant Foods Hum. Nutr.*, **54**(4): 353–362.

Albertini, M.V.; Carcouet, E.; Pailly, O.; Gambotti, C.; Luro, F. and Berti, L. (2006). Changes in Organic Acids and Sugars during Early Stages of Development

of Acidic and Acidless Citrus Fruit. *J. Agric. Food Chem.,* **54**(21): 8335–8339.

Altaf, N.; Khan, A. R.; and Hussain, J. (2008). Fruit variability in Kinnow mandarin (*Citrus reticulata*). *Pak. J. Bot*, **40**(2): 599-604.

Amorim, J. L.; Simas, D. L. R.; Pinheiro, M. M. G.; Moreno, D. S. A.; Alviano, C. S.; da Silva, A. J. R., and Dias Fernandes, P. (2016). Anti-inflammatory properties and chemical characterization of the essential oils of four *Citrus* species. *PloS one*, **11**(4): e0153643.

Ani, P. N. and Abel, H. C. (2018). Nutrient, phytochemical, and antinutrient composition of *Citrus* maxima fruit juice and peel extract. *Food Sci. Nutr.*, **6**(3): 653-658.

A.O.A.C (1970) Official method of Analysis. 10th Edition, *Association of Official Analytical Chemists*, Washington DC

A.O.A.C (1980) Official method of Analysis. 13th Edition, *Association of Official Analytical Chemists*, Washington DC.

A.O.A.C (2005) Official method of Analysis. 18th Edition, *Association of Official Analytical Chemists*, Washington DC.

Asencio, A. D.; Serrano, M.; García-Martínez, S. and Pretel, M. T. (2018). Organic acids, sugars, antioxidant activity, sensorial and other fruit characteristics of nine traditional Spanish *Citrus* fruits. *Eur. Food Res Technol.,* **244**: 1497-1508.

Asjad, H. M. M.; Akhtar, M. S.; Bashir, S.; Din, B.; Gulzar, F.; Khalid, R. and Asad, M. (2013). Phenol, flavonoid contents and antioxidant activity of six common *Citrus* plants in Pakistan. *J. Pharm. Sci.*, **1**(1): 1-5.

Atkins, C. D.; Wiederhold, E. and Moore, E. L. (1945). Vitamin C content of processing residue from Florida *Citrus* Waste. *Fruit Prod. J.*, **24**(9): 260-262.

Atolani, O.; Adamu, N.; Oguntoye, O. S.; Zubair, M. F.; Fabiyi, O. A. and Oyegoke, R. A., (2020). Chemical characterization, antioxidant, cytotoxicity, anti-toxoplasma gondii and antimicrobial potentials of the *Citrus sinensis* seed oil for sustainable cosmeceutical production. *Heliyon*, **6**(2): e03399.

Badiyala, S.D. and Sharma, S.K. (2004). Variability studies in galgal (*Citrus pseudolimon* Tanaka) in upper Shivaliks of Himachal Pradesh. *Indian J. Hortic*. **61**(2): 105-108.

Bae, J. M.; Lee, E. J. and Guyatt, G. (2009). *Citrus* fruit intake and pancreatic cancer risk: a quantitative systematic review. *Pancreas*, **38**(2): 168-174.

Baghurst, K. and CSIRO. (2003). Health Sciences and Nutrition and Horticulture Australia. *Update of citrus health benefits literature review*, CSIRO, Australia.

Bailey, M. I. and Thomas, A. W. (1942). The Thiamine and Riboflavin Contents of *Citrus* Fruits. *J. Nutr.*, **24**(1)*: 85–92.*

Bakkali, F.; Averbeck, S.; Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils–a review. *Food Chem. Toxicol.*, **46**: 446–475.

Baldwin, E. A. (2002). Fruit flavor, volatile metabolism and consumer perceptions. In: *Fruit quality and its biological basis*, 89-106.

Barbora, A. C.; Deka, S.; Kakoti, R. K.; Dutta, J. P.; Gogoi, A. and Saikia, J. (2020). Genetic diversity of *Citrus* Germplasm in Northeastern region of India. *Int. J. Adv. Biol. Res*, **10**: 53-61.

Barros, H. R.; de Castro Ferreira, T. A. P. and Genovese, M. I. (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of *Citrus* from Brazil. *Food chem.*, **134**(4): 1892-1898.

Basak, J. K.; Madhavi, B. G. K.; Paudel, B.; Kim, N. E. and Kim, H. T. (2022). Prediction of total soluble solids and pH of strawberry fruits using RGB, HSV and HSL colour spaces and machine learning models. *Foods*, **11(**14): 2086.

BeMiller, J. N. (2017). Carbohydrate Analysis. In: *Food Analysis*, Neilsen, S.S (Ed.). Food Science text series, Springer, Boston, pp *333–360.*

BeMiller, J.N. (2017). pH and titratable acidity. In: *Food analysis*, Neilsen, S.S (Ed.). Food Science text series, Springer, Boston, pp 389-406.

Berk, Z. (2016). Introduction: History, production, trade, and utilization. In: *Citrus Fruit Processing, Berk, Z., (Ed). Academic Press: San Diego, CA, USA*, 1-8.

Bharali, R.; Bhattacharyya, R. K. and Das, P. (2017). Bioactive compounds and total antioxidant activity of pummelo (*Citrus grandis* L.) ecotypes of Assam. *Bull. Environ. Pharm. Life Sci.*, **6**(1): 342-347.

Bhattacharya S.C. and Dutta S. (1956). Classification of *Citrus* fruits of Assam. Sc. Monogr. 20. ICAR, New Delhi, pp 110.

Bhattacharya, R.K. and Baruah, B.P. (1998). Citrus. In: *A textbook on Pomology, Vol. III*, Chatopadhyay, T.K. (Ed.), Kalyani Publishers, New Delhi: 1-71.

Bhuyan, N.; Barua, P. C.; Kalita, P. and Saikia, A. (2015). Physico-chemical variation in

peel oils of Khasi mandarin (*Citrus reticulata* Blanco) during ripening. *Indian J. Plant Physiol.*, **20**: 227-231.

Blanco, D.; Quintanilla, M. E.; Mangas, J. J. and Gutiérrez, M. D. (1996). Determination of organic acids in apple juice by capillary liquid chromatography. *J. Liq. Chromatogr Relat.*, **19**(16): 2615-2621.

Block, G. (1991). Vitamin C and cancer prevention: the epidemiologic evidence. *Am. J. Clin. Nutr.*, **53**(1): 270-282.

Bora, H.; Kamle, M.; Mahato, D. K.; Tiwari, P. and Kumar, P. (2020). *Citrus* Essential Oils (CEOs) and Their Applications in Food: An Overview. *Plants*, **9**(3): 357.

Bousbia, N.; Vian, M. A.; Ferhat, M. A.; Meklati, B. Y. and Chemat, F. (2009). A new process for extraction of essential oil from *Citrus* peels: Microwave hydrodiffusion and gravity. *J. food Eng.*, **90**(3): 409-413.

Braddock, R. J. (1972). Twenty-third *Citrus* Processors' Meeting Report; Agric. Res. Education Ctr.: Lake Alfred, Florida.

Caruso M.; Smith M. W.; Froelicher Y.; Russo G. and Gmitter F. G. (2020). Traditional breeding. In *The Genus Citrus*, Talon M., Caruso M., Gmitter F (Eds.). Elsevier Inc., Amsterdam: 129-148

Castle, W.S.; Baldwin, J.C. and Muraro, R.P. (2010). Rootstocks and the performance and economic returns of "Hamlin" sweet orange trees. *Hort. Sci*., **45**(6): 875–881.

Cebadera-Miranda, L.; Domínguez, L.; Dias, M. I.; Barros, L.; Ferreira, I.; Igual, M. and Cámara, M. (2019). Sanguinello and Tarocco (*Citrus sinensis* [L.] Osbeck): Bioactive compounds and colour appearance of blood oranges. *Food Chem.*, **270**: 395–402.

Cervantes, E.; Martín, J. J. and Saadaoui, E. (2016). Updated Methods for Seed Shape Analysis. *Scientifica*, **4**:1–10.

Chasapis C.T.; Loutsidou A.C.; Spiliopoulou C.A. and Stefanidou M.E. (2012). Zinc and human health: an update. *Arch Toxicol*, **86**:521– 534.

Chau, C. F. and Huang, Y. L. (2003). Comparison of the chemical composition and physicochemical properties of different fibres prepared from the peel of *Citrus sinensis* L. Cv. Liucheng. *J. Agric. Food Chem.*, **51**(9): 2615–2618.

Chen, W. and Viljoen, A. M. (2010). Geraniol — A review of a commercially important

fragrance material. *S Afr. J. Bot.,* **76**(4): 643-651.

Chesworth, J. M.; Stuchbury, T. and Scaife, J. R. (1998). The Carbohydrates. In: *An Introduction to Agricultural Biochemistry,* Springer, Dordrecht, pp 19-34.

Chinnici, F.; Spinabelli, U.; Riponi, C. and Amati, A. (2005). Optimization of the determination of organic acids and sugars in fruit juices by ion-exclusion liquid chromatography. *J. food Compos. Anal.*, **18**(2-3): 121-130.

Cirmi, S.; Navarra, M.; Woodside, J. V. and Cantwell, M. M. (2018). *Citrus* fruits intake and oral cancer risk: A systematic review and meta-analysis. *Pharmacol. Res.,* **133**: 187–194.

Clements, R. L. (1964). Organic acids in *Citrus* fruits. I. Varietal differences. *J. Food Sci.*, **29**(3): 276-280.

Clements, R. L. (1964). Organic Acids in *Citrus* Fruits. II. Seasonal Changes in the Orange. *J. Food Sci.,* **29**(3): 281-286.

Cohen, I.; Daut, J. and Noble, D. (1976). The effects of potassium and temperature on the pace-maker current, iK2, in Purkinje fibres. *J. Physiol.*, **260**(1): 55-74.

Colditz, G. A.; Stampfer, M. J. and Willett, W. C. (1987). Diet and lung cancer: a review of the epidemiologic evidence in humans. *Arch. Intern. Med*, **147**(1): 157-160.

Cronje, P. J.; Stander, O. P. and Theron, K. I. (2013). Fruit splitting in citrus. *Hortic. Rev.*, **41**:177-200.

Curl, A. L. and Bailey, G. F. (1954). Orange Carotenoids, Polyoxygen Carotenoids of Valencia Orange Juice. *J. Agric. Food Chem.,* **2**(13): 685-690.

Czech, A.; Zarycka, E.; Yanovych, D.; Zasadna, Z.; Grzegorczyk, I. and Kłys, S. (2020). Mineral content of the pulp and peel of various *Citrus* fruit cultivars. *Biol. Trace Elem. Res.*, **193**: 555-563.

Ornelas-Paz, J.; Yahia, E.M.; Ramírez-Bustamante, N.; Pérez-Martínez, J.D.; del Pilar Escalante-Minakata, M.; Ibarra-Junquera, V.; Acosta-Muñiz, C.; Guerrero-Prieto, V.; Ochoa-Reyes, E. (2013). Physical Attributes and Chemical Composition of Organic Strawberry Fruit (*Fragaria x ananassa* Duch, Cv. Albion) at Six Stages of Ripening. *Food Chem.,* **138:** 372–381.

Dalal, R.;Beniwal, B. and Sehrawat, S.K. (2013). Seasonal Variation in Growth, Leaf Physiology and Fruit Development in Kinnow, a Mandarin Hybrid. *J. Plant Stud.,*

2: 72-77.

Debbabi, O. S.; Bouhlal, R.; Abdelaali, N.; Mnasri, S. and Mars, M. (2013). Pomological study of sweet orange (*Citrus sinensis* L. Osbeck) cultivars from Tunisia. *Int. J. Fruit Sci.*, **13**(3): 274-284.

Deshmukh, N. A.; Patel, R. K.; Rymbai, H.; Jha, A. K. and Deka, B. C. (2016). Fruit maturity and associated changes in Khasi mandarin (*Citrus reticulata*) at different altitudes in humid tropical climate. *Indian J. Agric. Sci*, **86**(7): 854-859.

Dey, K. (2016) Influence of the tree canopy positions and directions on fruit morphological characters and bio-chemical composition of pummelo (*Citrus grandis* Osbeck.). *Ecosan.*, **19:**1-8.

Deyhim F.; Lopez E.; Gonzalez J.; Garcia M. and Patil, B.S. (2006) *Citrus* juice modulates antioxidant enzymes and lipid profiles in orchidectomized rats. *J. Med. Food*, **9**:422–426.

Diwan, K.; Naik, D.M.; Y. Vadak; Taru A.S. and B.K. Maholiya. (2014). Variability, correlation and path analysis studies for superior types of sweet orange (*Citrus sinensis* Osbeck). *Int. J. of Agric. Sci.***, 10**(2): 649-653.

Dorji, K. and Yapwattanaphun, C. (2011). Morphological identification of mandarin (*Citrus reticulata* Blanco) in Bhutan. *Agric. Nat, Resour.*, **45**(5): 793-802.

Duarte, A. M.; Caixeirinho, D.; Miguel, M. G.; Sustelo, V.; Nunes, C.; Fernandes, M. M. and Marreiros, A. (2010). Organic acids concentration in *Citrus* juice from conventional versus organic farming. *Acta Horticulturae.*, **933**: 601-606.

Dubey, A.K. (2000). Comparative performance of certain sweet orange (*Citrus sinensis* Osbeck) cultivars at foothills situation of Arunachal Pradesh. *Progress. Hortic.*, **33**(2):153-158.

Dziezak, J. D. (2016). Acids: Natural Acids and Acidulants. In: *Encyclopedia of Food and Health*, Caballero, B., Finglas, P.M., Toldra, F. (Eds.). Academic Press. pp15–18.

Ebrahimzadeh M.A.; Hosseinimehr S.J. and Gayekhloo M.R. (2004). Measuring and comparison of vitamin C content in *Citrus* fruits: introduction of native variety. *Indian J. Chem.*, **1**(9): 650-652.

El-Beltagi, H. S.; Eshak, N. S.; Mohamed, H. I.; Bendary, E. S. A. and Danial, A. W. (2022). Physical Characteristics, Mineral Content, and Antioxidant and Antibacterial

Activities of *Punica granatum* or *Citrus sinensis* Peel Extracts and Their Applications to Improve Cake Quality. *Plants*, **11**(13): 1740.

Ergönül, P. G. and Nergiz, C. (2010). Determination of organic acids in olive fruit by HPLC. *Czech. J. Food. Sci.*, **28**(3): 202-205.

Erkan, M.; Dogan, A. (2019). Harvesting of horticultural commodities. In Postharvest Technology of Perishable Horticultural Commodities; Woodhead Publishing: Cambridge, UK, 2019; pp. 129–159.

Evans, R.H.; Van Soestbergen, A.W. and Ristow, K.A. (1983). Evaluation of apple juice authenticity by organic acid analysis. *J. Assoc. Off. Anal. Chem.,* **66**: 1517-1520.

Ezeabara, C. A.; Okeke, C. U.; Aziagba, B. O.; Ilodibia, C. V. and Emeka, A. N. (2014). Determination of saponin content of various parts of six *Citrus* species. *Int. Res. J. Pure Appl. Chem.*, **4**(1): 137.

Ezeabara, C.A; Okeke, C.U. and Aziagba, B.O. (2013). Flavonoid content of Citrus Species grown in Awka, Anambra State, Southeastern Nigeria. *Inter J Agri Biosci*., **2**(3): 103-107.

Fallahi, E.; Rodney, D. R. and Mousavi, Z. (1990). Growth, yield, and fruit quality of eight lemon cultivars in Arizona. *J. Am. Soc. Hortic. Sci.*, **115**(1): 6-8.

Fang, T.; Goto, M.; Sasaki, M. and Hirose, T. (2004). Combination of supercritical CO2 and vacuum distillation for the fractionation of bergamot oil. *J. Agric. Food Chem.,* **52**: 5162–5167.

Fatta Del Bosco, S.; Matranga, G. and Geraci, G. (1992). Micro and macro-sporogenesis of two triploid hybrids of citrus. In *Proceedings of 7th international citrus congress*, **1**: 122-124.

Ferhat, M. A.; Meklati, B. Y.; Smadja, J. and Chemat, F. (2006). An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. *J. Chromatogr. A*, **1112**(1-2): 121-126.

Fernandez-Lopez, J.; Zhi, N.; Aleson-Carbonell, L.; Pérez-Alvarez, J. A. and Kuri, V. (2005). Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat sci.*, **69**(3): 371-380.

Foschi, R.; Pelucchi, C.; Dal Maso, L.; Rossi, M.; Levi, F.; Talamini, R.; Bosetti, C.; Negri, E.; Serraino, D.; Giacosa, A. and Franceschi, S (2010). *Citrus* fruit and cancer risk

in a network of case–control studies. *Cancer causes control*, **21**: 237-242.

Freed, K. F. (1966). Theory of the hyperfine structure of molecules: Application to 3π states of diatomic molecules intermediate between Hund's Cases (a) and (b). *J Chem. Phys.*, **45**(11): 4214-4241.

Frometa, E. and Echazabal, J. (1988). Influence of age and cultivar on juice characteristics of early oranges. *Agrotecnia Cuba*., **20**: 71–75.

Fuster, M. D. (1997). *Citrus Flavonoids. Distribution, modulation by phytorregulators and their possible physiological function*. PhD. University of Murcia. Spain.

Future market insight report (2021) Citrus oils market outlook. https://www.futuremarketinsights.com/reports/*Citrus*-oils-market) retrieved on 11 April, 2023.

Gaikwad K. A.; Haldavnekar P. C.; Parulekar, Y. R. and P. M. Haldankar (2015). Survey and characterization of pummelo genotypes (*Citrus grandis*. Osbeck) grown in coastal region of Maharashtra. *The Ecoscan*, **8**: 371-380.

Gaikwad, K. A.; Patil, S. R.; Nagre, P. K. and Potdukhe, N. R. (2018). Morphological characterization of *Citrus* rootstock genotypes. *Int. J. Chem. Stud.*, **6**(2): 516-529.

George, A. P.; Broadley, R. H.; Nissen, R. J.; Hamill, S. D. and Topp, B. L. (1999). Breeding strategies for atemoya and cherimoya. *Acta Hortic*. **497:** 255-268.

Ghani, A.; Mohtashami, S. and Jamalian, S. (2021). Peel essential oil content and constituent variations and antioxidant activity of grapefruit (*Citrus*× *paradisi* var. red blush) during colour change stages. *J. Food Meas. Charact.*, **15**: 4917-4928.

Giménez-Sanchis, A.; Pons-Gómez, A.; Farina, V. and Besada, C. (2022). Effect of Pulp Pigmentation Intensity on Consumer Acceptance of New Blood Mandarins: A Cross-Cultural Study in Spain and Italy. *Agronomy*, **12**(12): 3058.

Goldschmidt, E. E. and Koch, K. E. (1996). Citrus. In: *Photoassimilate Distribution in Plants and Crops* (Zamski, E. and SchaVer, A.A.) (Eds.). Marcel Dekker Inc., New York, USA, pp. 797–824.

Gonzalez, C.A.; Lujan-Barroso, L.; Bueno-de-Mesquita, H.B.A.; Jenab, M.; Duell, E.J.; Agudo, A.; Tjønneland, A.; Boutron-Ruault, M.C.; Clavel-Chapelon, F.; Touillaud, M. and Teucher, B. (2012). Fruit and vegetable intake and the risk of gastric adenocarcinoma: a reanalysis of the European Prospective Investigation into

Cancer and Nutrition (EPIC-EURGAST) study after a longer follow-up. *Int. J. Cancer*, **131**(12): 2910-2919.

Gorinstein, S.; Cvikrová, M.; Machackova, I.; Haruenkit, R.; Park, Y.S.; Jung, S.T.; Yamamoto, K.; Ayala, A.L.M.; Katrich, E. and Trakhtenberg, S. (2004). Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food chem.*, **84**(4): 503-510.

Govind, S., and Singh, I. P. (1999). Effect of foliar application of urea, GA_3 and $ZnSO_4$ on seedling growth of two *Citrus* species. *Jour. Appl. Hort.*, **1**(1): 51-53.

Govind, S., and Singh, I.P. (2002). Evaluation of *Citrus* germplasm in north-eastern hill region. *Indian J. Plant Genet. Resour.*, **15**: 19-22.

Graebin, C. S.; Madeira, M. D. F.; Yokoyama-Yasunaka, J. K.; Miguel, D. C.; Uliana, S. R.; Benitez, D. and Eifler-Lima, V. L. (2010). Synthesis and in vitro activity of limonene derivatives against Leishmania and Trypanosoma. *Eur. J. Med. Chem.*, **45**(4): 1524-1528.

Grosso, G.; Bei, R.; Mistretta, A.; Marventano, S.; Calabrese, G.; Masuelli, L.; Giganti, M.G.; Modesti, A.; Galvano, F. and Gazzolo, D. (2013). Effects of vitamin C on health: a review of evidence. *Front Biosci. (Landmark Ed)*, **18**(3): 1017-1029.

Gupta, A. K.; Mishra, P.; Senapati, M. and Sahu, P. P. (2021). A novel electrochemical device for naringin quantification and removal from bitter variety of *Citrus* fruits. *Jour. Food Eng.*, **306**: 110637.

Hamdan, D.; El-Readi, M. Z.; Nibret, E.; Sporer, F.; Farrag, N.; El-Shazly, A. and Wink, M. (2010). Chemical composition of the essential oils of two *Citrus* species and their biological activities. *Pharmazie*, **65**(2): 141-147.

Hangsing, H.; Mathew, B. and Kalita, D. C. (2016). Performance of Khasi mandarin in Garo Hills of Meghalaya. *Int. Jour. Sci. Env. Tech.*, **5**(5): 3213-3223.

Haque, M. N.; Saha, K.; Karim, M. R. and Bhuiyan, M.N.H. (2009). Evaluation of nutritional and physico-chemical properties of several selected fruits in Bangladesh. *Bangladesh J. Sci. Ind. Res.*, **44**(3): 353-358.

Harris, G. K., and Marshall, M. R. (2017). *Ash Analysis. Food Analysis*, *287–297.*

Hasib, A.; Jaouad, A.; Mahrouz, M. and Khouili, M. (2002). HPLC determination of organic acids in Moroccan apricot. *CYTA-J. Food*, **3**(4): 207-211.

Hassan, N. A.; A El-Halwagi, A.; Khalaf, R. M.; Sayed, H. A. and El-Homosany, A. A. (2008). Morphological characterization, pollen grain fertility and some chemical characters of selected mandarin (*Citrus* Spp.) varieties. *Arab Univ. J. Agri. Sci.*, **16**(1): 161-177.

Hazarika, T. K. (2012). *Citrus* genetic diversity of north-east India, their distribution, ecogeography and ecobiology. *Genet. Resour. Crop evol.,***59***(6):*1267-1280.

Henneberg, W. (1865). Die Salzmiinder Fiitterungsversuche. *J. Landwirtschaft. Heft*, **1**: 89-192.

Herath, H. M. P. D.; Dissanayake, M. D. M. I. M.; Dissanayake, D. R. R. P.; Chamikara, M. D. M.; Kularathna, K. W. T. R.; Ishan, M. and Sooriyapathirana, S. D. S. S. (2016). Assessment of the variations in selected industrially desirable morphological and biochemical traits of eleven *Citrus* species in Sri Lanka. *Procedia. Food Sci.*, **6**: 176-180.

Hodges, R. E.; Baker, E. M.; Hood, J.; Sauberlich, H. E. and March, S. C. (1969). Experimental scurvy in man. *Am. J. of Clin. Nutr.*, **22**(5): 535-548.

Hodgson, R.W. (1965). Taxonomy and nomenclature in *Citrus* fruits. In: Advances in agricultural sciences and their applications. Krishnamurthi S (ed.). Madras Agric J, Madras, pp 317–331.

Hodgson, R.W. (1967). The horticultural varieties of citrus. In: The *Citrus* industry, Reuther, W., Webber, H.J. and Batchelor, L.D. (Eds.). University of California, Division of Agricultural Sciences, USA, pp. 431-591.

Hore, D. K. and Barua, U. (2004). Status of citriculture in North Eastern region of India–A review. *Agricultural Reviews*, **25**(1): 1-15.

Hou, H. S.; Bonku, E. M.; Zhai, R.; Zeng, R.; Hou, Y. L.; Yang, Z. H. and Quan, C. (2019). Extraction of essential oil from *Citrus reticulate* Blanco peel and its antibacterial activity against *Cutibacterium acnes* (formerly *Propionibacterium acnes*). *Heliyon*, **5**(12): e02947.

Huet, R. (1991). Les huiles essentielles d'agrumes (suite). *Fruits*, **46**(5): 551-576.

Hyodo, H. (1981). Ethylene production by citrus fruit tissues. *Proc. Int. Soc. Citric.*, Tokyo, Japan, pp. 880–882.

ICMR (2010). Nutrient requirements and recommended dietary allowances for Indians. A

report of the expert group of the Indian Council of Medical Research. New Delhi.

Ifmalinda; Putri, R. E. and Rasinta, I. (2022). Estimation of Size, Volume and Weight of Oranges Using Digital Images Processing. In *IOP Conference Series: Earth and Environmental Science*., **1059**: 012016.

Iglesias, D. J.; Tadeo, F. R.; Primo-Millo, E. and Talon, M. (2006). Carbohydrate and ethylene levels regulate citrus fruitlet drop through the abscission zone A during early development. *Trees—Struct. Func.*, **20**: 348–355.

Ikeda, R. M.; Stanley, W. L.; Rolle, l. A. and Vannier, S. H. (1962). Monoterpene Hydrocarbon Composition of *Citrus* Oils. *J. Food Sci.*, **27**(6): 593-596.

Indulekha, J.; Gokul Siddarth, M. S.; Kalaichelvi, P. and Arunagiri, A. (2017). Characterization of *Citrus* Peels for Bioethanol Production. *Int. J Energy Env. Eng.*, **1**:3–12.

IPGRI (1999). Descriptors for Citrus. International Plant Genetic Resource Institute. Rome, Italy.

Iqbal, K.; Khan, A.; and Khattak, M. M. A. K. (2004). Biological significance of ascorbic acid (vitamin C) in human health-a review. *Pak. J. Nutr.*, **3**(1): 5-13.

Iqbal, S. M.; Gopal, A.; Sankaranarayanan, P. E. and Nair, A. B. (2016). Classification of selected citrus fruits based on color using machine vision system. *Int. J. Food Prop.*, **19**(2): 272-288.

Jamil, N.; Jabeen, R.; Khan, M.; Riaz, M.; Naeem, T.; Khan, A.; Sabah, N.U.; Ghori, S.A.; Jabeen, U.; Bazai, Z.A.; Mushtaq, A. and Fahmid, S., (2015). Quantitative assessment of juice content, citric acid and sugar content in oranges, sweet lime, lemon and grapes available in fresh fruit market of Quetta city. *Int. J. Basic Appl. Sci.*, **15**(1): 21-24.

Jaskani, M. J.; Abbas, H.; Khan, M. M.; Shahzad, U. and Hussain, Z. (2006). Morphological description of three potential *Citrus* rootstocks. *Pak. J. Bot.*, **38**(2): 311.

Javed, S.; Ahmad, R.; Shahzad, K.; Nawaz, S.; Saeed, S. and Saleem, Y. (2013). Chemical constituents, antimicrobial and antioxidant activity of essential oil of *Citrus limetta* var. Mitha (sweet lime) peel in Pakistan. *Afr. J. Microbiol. Res.*, **7**(24): 3071-3077.

Javed, S.; Javaid, A.; Nawaz, S.; Saeed, M. K.; Mahmood, Z.; Siddiqui, S. Z. and Ahmad, R. (2014). Phytochemistry, GC-MS analysis, antioxidant and antimicrobial

potential of essential oil from five *Citrus* species. *J. Agric. Sci.*, **6**(3): 201.

Jing, L.; Lei, Z.; Li, L.; Xie, R.; Xi, R.; Xi, W.; Guan, Y.; Summer, L. and Zhou, Z. (2014). Antifungal activity of citrus essential oils. *J. Agric. Food Chem*., **62**: 3011–3033.

Jirovetz, L.; Buchbauer, G.; Stoyanova, A. S.; Georgiev, E. V. and Damianova, S. T. (2005). Composition, quality control and antimicrobial activity of the essential oil of cumin (*Cuminum cyminum* L.) seeds from Bulgaria that had been stored for up to 36 years. *Int. J. food Sci. Technol.*, **40**(3): 305-310.

Johnson, R. S. (1996). Key Nutrients. Cooperative Extension Service. *Iowa State University of Science and Technology, Ames, Iowa*.

Josan, J. S. and Kaur, N. (2004). Variability and Character Association Analysis in Sweet Orange (*Citrus sinensis*) Varieties. *Indian J. Plant Genet. Resour.*, **17**(3): 175-177.

Josan, J.S. and Kaur, N. (2006). Variability and character association analysis in identified mandarin germplasm. *Indian J. Hortic.*, **63**(2): 152-154.

Joseph, G. (2016). Physicochemical and Proximate Analysis of Extracts from *Citrus sinensis* of Dutsinma, Katsina State, Nigeria. *Open Access Libr. J.*, **3**(03): 1.

Kahn, T. L. and Vidalakis, G. (2014). Scion cultivars. *Citrus production manual.* UCANR Publications, Richmond, 63-94.

Kakoti, R. K.; Saikia, J.; Deka, S.; Gogoi, A. and Barbora, A. C. (2019). Present Status of Khasi Mandarin in Manipur State of North East India. *Int. J. Curr. Microbiol. Appl. Sci.*, **8**: 2157-2165.

Kakoti, R. K.; Saikia, J.; Deka, S.; Gogoi, A. and Barbora, A. C. (2019). Present status of Khasi Mandarin in Tripura state of North East India. *Int. J. of Chem. Stud.*, **7**(4): 2943-2948.

Kamal, G. M.; Ashraf, M. Y.; Hussain, A. I.; Shahzadi, A. and Chughtai, M. I. (2013). Antioxidant potential of peel essential oils of three Pakistani *Citrus* species: *Citrus reticulata*, *Citrus sinensis* and *Citrus paradisii*. *Pak. J. Bot.*, **45**(4): 1449-1454.

Kanaze, F. I.; Termentzi, A.; Gabrieli, C.; Niopas, I.; Georgarakis, M. and Kokkalou, E. (2009). The phytochemical analysis and antioxidant activity assessment of orange peel (*Citrus sinensis*) cultivated in Greece–Crete indicates a new commercial source of hesperidin. *Biomed. Chromatogr.*, **23**(3): 239-249.

Kapoor, L.; Simkin, A. J.; George Priya Doss, C. and Siva, R. (2022). Fruit ripening:

dynamics and integrated analysis of carotenoids and anthocyanins. *BMC plant Biol.*, **22**(1): 27.

Karadeniz, F. (2004). Main organic acid distribution of authentic *Citrus* juices in Turkey. *Turk. J. Agric. For.*, **28**(4): 267-271.

Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M.; Koizumi, M.; Ito, C. and Furukawa, H. (2000). Quantitative study of flavonoids in leaves of citrus plants. *J. agric. food chem.*, **48**(9):3865–3871.

Kayesh, E.; Das, S.; Roni, M. S.; Rajib, M. M. R.; Islam, M. M. and Sultana, H. (2018). Shelf life and quality of Sorboti lemon as affected by different chemicals and storage temperature. *AAES*, **3**(3): 303-309.

Kelebek, H. (2010). Sugars, organic acids, phenolic compositions and antioxidant activity of Grapefruit (*Citrus paradisi*) cultivars grown in Turkey. *Ind. Crops Prod.*, **32**(3): 269-274.

Kennedy, D.O. (2016). B vitamins and the brain: mechanisms, dose and efficacy-A review. *Nutrients*, **8**(2): 1–29.

Khadivi-Khub, A. (2013). Analysis of Some Technological and Physical Characters of Mandarin (Citrus reticulata) Fruit in Iran. *Int. Sco. Res. Notices, **2013***: 1-4.

Khalid, S.; Malik, A. U.; Khan, A. S. and Jamil, A. (2012). Influence of exogenous applications of plant growth regulators on fruit quality of young kinnow mandarin (*Citrus nobilis*× *C. deliciosa*) trees. *International Journal of Agriculture and Biology*, **14**(2): 229-234.

Khan, A.; Hussain, S.A.; Nabi, G. and Asghar, H. (2005). Screening of sweet orange Varieties on physico-chemical characters. *Sarhad J. Agric*., **21** (2): 205-208.

Khan, M. K.; Zill-E-Huma, and Dangles, O. (2014). A comprehensive review on flavanones, the major *Citrus* polyphenols. *J. Food Compost. Anal.*, **33**(1): 85–104.

Khan, M. N.; Nawaz, M. A.; Ahmad, W.; Afzal, M.; Malik, A. U. and Saleem, B. A. (2010). Evaluation of some exotic cultivars of sweet orange in Punjab, Pakistan. *Int. J. Agric. Biol*, **12**: 729-733.

Kim, M. J.; Jung, H. N.; Kim, K. N. and Kwak, H. K. (2008). Effects of cranberry powder on serum lipid profiles and biomarkers of oxidative stress in rats fed an atherogenic diet. *Nutr Res. Pract.*, **2**(3): 158-164.

Kim, S.S.; Baik J.S.; Oh, T.H.; Yoon, W.J.; Lee, N.H.; Hyun, C.G. (2008). Biological activities of Korean *Citrus obovoides* and *Citrus natsudaidai* essential oils against acne-inducing bacteria. *Biosci. Biotechnol. Biochem.*, **72**:2507–2513.

Kopustinskiene, D. M.; Jakstas, V.; Savickas, A. and Bernatoniene, J. (2020). Flavonoids as anticancer agents. *Nutrients*, **12**(2): 457.

Koshita, Y. (2015). Effect of temperature on fruit colour development. In: *Abiotic stress biology in horticultural plants*, Kanayama, Y. and Kochetov, A. (Eds.). Springer Japan.

Kostadinović, S.; Jovanov, D. and Mirhosseini, H. (2014). Comparative investigation of cold pressed essential oils from peel of different mandarin varieties. *The IIOAB J.*, **3**: 7–14.

Kumar, A.; Avasthe, R. K.; Pandey, B.; Lepcha, B. and Rahman, H. (2011). Effect of fruit size and orchard location on fruit quality and seed traits of mandarin (*Citrus reticulata*) in Sikkim Himalayas. *Indian J. of Agric. Sci.*, **81**(9): 821.

Kumar, C. S.; Ali, A. and Manickavasagan, A. (2020). Health benefits of substituting added sugars with fruits in developing value-added food products: a review. *Intr. J. Nutr. Pharmacol. Neurol. Dis.*, **10**(3): 75-90.

Ladaniya, M.S. (2008). *Citrus Fruit: Biology, Technology and Evaluation*. Academic Press, San Diego, California, USA. pp 1-11: 573.

Lan phi, N. T.; Nishiyama, C.; Choi, H.S.; and Sawamura, M. (2006). Evaluation of Characteristic Aroma Compounds of *Citrus natsudaidai* Hayata (Natsudaidai) Cold-Pressed Peel Oil. *Biosci. Biotechnol. Biochem.*, **70**(8)*:* 1832–1838.

Lee, S. K. and Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest boil. technol.*, **20**(3): 207-220.

Li, C. and Schluesener, H. (2017). Health-promoting effects of the *Citrus* flavanone hesperidin. *Crit. Rev. Food Sci. Nutr.*, **57**(3): 613-631.

Li, G.X. and Liu, Z.Q. (2009). Unusual antioxidant behaviour of alpha- and gamma-terpinene in protecting methyl linoleate, DNA, and erythrocyte. *J. Agric. Food Chem.*, **57**: 3943–3948.

Li, L., and Sheen, J. (2016). Dynamic and diverse sugar signaling. *Curr. Opin. Plant Biol.*, **33**: 116–125.

Liu, F.; Osman, A., Yusof, S. and Ghazali, H. M. (2005). Effects of enzyme-aided peeling on the quality of local mandarin (*Citrus Reticulata*) Segments. J. Food Process. Preserv., **28**(5): 336–347.

Liu, K.; Wang, X.; Luo, B.; Wang, C.; Hou, P.; Dong, H. and Zhao, C. (2022). Enzyme-Free Electrochemical Sensors for In Situ Quantification of Reducing Sugars Based on Carboxylated Graphene–Carboxylated Multiwalled Carbon Nanotubes–Gold Nanoparticle–Modified Electrode. *Front Plant Sci.*, **13**: 872190.

Liu, N.; Li, X.; Zhao, P.; Zhang, X.; Qiao, O.; Huang, L.; Guo, L. and Gao, W. (2021). A review of chemical constituents and health-promoting effects of *Citrus* peels. *Food Chem.*, **365**: 130585.

Liu, X.; Li, J.; Huang, M. and Chen, J. (2015). Mechanisms for the Influence of Citrus Rootstocks on Fruit Size. *J. Agric. Food Chem.*, **63**(10): 2618-2627.

Liu, Y.; Heying, E. and Tanumihardjo, S. A. (2012). History, Global Distribution, and Nutritional Importance of Citrus Fruits. *Compr. Rev. Food Sci. Food Saf.*, **11**(6): 530–545.

Lota, M.-L.; de Rocca Serra, D.; Tomi, F.; Casanova, J. (2000). Chemical variability of peel and leaf essential oils of mandarins from Citrus reticulata Blanco. *Biochem. Syst. Ecol.*, **28**: 61–78.

Lu, Q.; Huang, N.; Peng, Y.; Zhu, C. and Pan, S. (2019). Peel oils from three *Citrus* species: volatile constituents, antioxidant activities and related contributions of individual components. *J. food sci. tech.*, **56**(10): 4492–4502.

Lu, X.; Zhao, C.; Shi, H.; Liao, Y.; Xu, F.; Du, H.; Xiao, H. and Zheng, J. (2023). Nutrients and bioactives in *Citrus* fruits: Different *Citrus* varieties, fruit parts, and growth stages. *Crit. Rev. Food Sci. Nutr.*, **63**(14): 2018-2041.

Mahmoud, A. M.; Hernandez Bautista, R. J.; Sandhu, M. A. and Hussein, O. E. (2019). Beneficial effects of *Citrus* flavonoids on cardiovascular and metabolic health. *Oxidative Med. Cell. Longev.*, **19:** 5484138.

Malhotra, S.; Suri, S. and Tuli, R. (2008). Antioxidant activity of citrus cultivars and chemical composition of *Citrus karna* essential oil. *Planta medica*, **75**(1): 62-64.

Malik, S.K.; R. Chaudhury; O.P. Dhariwal and R.K. Kalia. (2006). Collection and characterization *of Citrus indica* Tanaka and *C. macroptera* Montr.: wild endangered species of northeastern India. *Genet. Resour. Crop Evol*., **53**(7): 1485-

1493.

Mansour, R. (2019). Determination of nutritional composition in *Citrus* fruits (*C. aurantium*) during maturity. *Nutr. Food Sci.*, **49**(2): 299-317.

Marboh, E. S.; Singh, A. K.; Dubey, A. K. and Prakash, J. (2015). Analysis of genetic variability among *Citrus* (*Citrus* spp) genotypes using morphological traits. *Indian J. Agric. Sci.*, **85**(2): 203-211.

Marsh, K. B.; Richardson, A. C. and Erner, Y. (2000). Effect of environmental conditions and horticultural practices on citric acid content. In: *Proc. Intl. Soc. Citricult. IX Congress*, pp 640-643.

Marshall, M. R. (2010). Ash Analysis. In: *Food Analysis*, Neilsen, S.S (Ed.). Food Science text series, Springer, Boston, pp *105–115.*

Matheyambath, A.C.; Padmanabhan, P. and Paliyath, G. (2016). Citrus fruits. In: *Encyclopedia of Food and Health*. University of Guelp. Guelp, Canada. pp. 136-140.

Mathias, D. J.; Kumar, S. and Rangarajan, V. (2019). An investigation on *Citrus* peel as the lignocellulosic feedstock for optimal reducing sugar synthesis with an additional scope for the production of hydrolytic enzymes from the aqueous extract waste. *Biocatal. Agric. Biotechnol.*, **20***:*101259.

Matsuo, Y.; Miura, L. A.; Araki, T. and Yoshie-Stark, Y. (2019). Proximate composition and profiles of free amino acids, fatty acids, minerals and aroma compounds in *Citrus natsudaidai* peel. *Food chem.*, **279**: 356-363.

Mehmood, T.; Afzal, A.; Anwar, F.; Iqbal, M.; Afzal, M.; Qadir, R. (2019). Variations in the Composition, Antibacterial and Haemolytic Activities of Peel Essential Oils from Unripe and Ripened *Citrus limon* (L.) Osbeck Fruit. *J. Essent. Oil Bear. Plants*, **22**:159–168.

Meneguzzo, F.; Ciriminna, R.; Zabini, F. and Pagliaro, M. (2020). Review of evidence available on hesperidin-rich products as potential tools against COVID-19 and hydrodynamic cavitation-based extraction as a method of increasing their production. *Processes*, **8**(5): 549.

Merle, H.; Morón, M.; Blázquez, M. A. and Boira, H. (2004). Taxonomical contribution of essential oils in mandarin cultivars. *Biochem. Syst. Ecol.*, **32**(5): 491-497.

M'hiri, N.; Ioannou, I.; Ghoul, M. and Mihoubi, N. B. (2015). Proximate chemical composition of orange peel and variation of phenols and antioxidant activity during convective air drying. *J. New Sci.*, **9**: 881–890.

Millar, C. L.; Duclos, Q. and Blesso, C. N. (2017). Effects of dietary flavonoids on reverse cholesterol transport, HDL metabolism, and HDL function. *Adv. Nutr.*, **8**(2): 226-239.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Anal. Chem*., **31:** 426–428.

Minh Tu, N. T.; Onishi, Y.; Choi, H. S.; Kondo, Y.; Ukeda, H. and Sawamura, M. (2003). Characteristic odour components of *Citrus* sp. (Kiyookadaidai) cold-pressed peel oil. *Flavour Fragr. J.*, **18**(6): 515-520.

Mohammed, A. M.; Alrayeh, A. S.; Mohamed, M. E.; and Abdel-Rahman, N. A. (2021). GC–MS of essential oil, metal profile and physicochemical properties of fruits of *Citrus macrophylla* Wester from Sudan. *Bull. Natl. Res. Cent.*, **45**: 1-8.

Mohammed, A.M.A.; Ibrahim, A.M.; Omran A.A.; Mohamed, M.E. and S.E.M. Elsheikh. (2013). Minerals content, essential oils composition and physicochemical properties of *Citrus jambhiri* Lush. (Rough Lemon) from the Sudan. *Int. Lett. Chem. Phys. Astron.*, **9**(1): 25-30.

Molyneux, R. J.; Lee, S. T.; Gardner, D. R.; Panter, K. E. and James, L. F. (2007). Phytochemicals: The good, the bad and the ugly? *Phytochem.*, **68**(22–24): 2973–2985.

Moosavy, M. H.; Hassanzadeh, P.; Mohammadzadeh, E.; Mahmoudi, R.; Khatibi, S. A. and Mardani, K. (2017). Antioxidant and antimicrobial activities of essential oil of Lemon (*Citrus limon*) peel in vitro and in a food model. *J. Food Qual. Hazards Control*, **4**(2): 42-48.

Morgan, K. T.; Rouse, R. E.; Roka, F. M.; Futch, S. H., and Zekri, M. (2005). Leaf and fruit mineral content and peel thickness of' Hamlin'orange. *Proc. annu. meet Fla.*, **118**: 19-21.

Mukhopadhyay, M. (2000). *Natural extracts using supercritical carbon dioxide*. CRC press.

Multari, S.; Licciardello, C.; Caruso, M. and Martens, S. (2020). Monitoring the changes in phenolic compounds and carotenoids occurring during fruit development in the tissues of four citrus fruits. *Food Res. Int.*, **134**: 109228.

Nagy, S, Chen C.S. and Shaw, P.E. (1993). Fruit Juice Processing Technology. Agscience Inc., Auburndale, FL, p 655.

Nair, K. N. and Nayar, M. P. (1997). Rutaceae. In: *Flora of India*, Hajra, P.K., Nair, V.J., Daniel, P. (Eds.). Botanical Survey of India: Calcutta, 229-407.

Nasir, M.; Akhtar, S. and Sharif, M. K. (2004). Effect of moisture and packaging on the shelf life of wheat flour. *J. Food Saf.* **4**:1-6.

Ncama, K.; Opara, U. L.; Tesfay, S. Z.; Fawole, O. A. and Magwaza, L. S. (2017). Application of Vis/NIR spectroscopy for predicting sweetness and flavour parameters of 'Valencia'orange (*Citrus sinensis*) and 'Star Ruby'grapefruit (*Citrus x paradisi* Macfad). *J. Food Eng.*, **193**: 86-94.

Nordby, H.E. and McDonald, R.E. (1990). Squalene in grapefruit wax as a possible natural protectant against chilling injury. *Lipids*, **25**:807–10.

Okwu, D. E. (2008). Citrus fruits: A rich source of phytochemicals and their roles in human health. *Int. J. Chem. Sci*, **6**(2): 451-471.

Olabinjo, O. O.; Ogunlowo, A. S.; Ajayi, O. O. and Olalusi, A. P. (2017). Analysis of physical and chemical composition of sweet orange (*Citrus sinensis*) peels. *Int. J. Environ. Agric. Biotechnol.*, **2**: 2201-2206.

Osarumwense, P. O.; Okunrobo, L. O. and Uwumarongie-Ilori, E. G. (2013). Phytochemical screening, proximate and elemental analysis of *Citrus sinensis* peels (L.) Osbeck. *J. Appl. Sci. Environ. Manag.*, **17**(1): 47-50.

P. S. Raju and A. S. Bawa (2012) Food Additives in Fruit Processing. In: *Handbook of Fruits and Fruit Processing*, Sinha, N; Sidhu, J.S.; Barta, J.; Wu, S. B.J. and Cano, M.P. (Eds.). John Wiley and Sons, Ltd., New Delhi, pp 189-228.

Palmer, J. K. and D.M. List. (1973). Determination of organic acids in foods by liquid chromatography. *J. Agric. Food Chem.*, **21**: 903- 906.

Park, H. R. and Shin, K. S. (2021). Inhibitory effects of orally administered pectic polysaccharides extracted from the *Citrus* Hallabong peel on lung metastasis. *Food Biosci.*, **43**: 101301.

Pascual, J. M. and Carmona, J. F. (1980). Composition of *Citrus* pulp. *Anim. Feed Sci, Technol.*, **5**(1): 1–10.

Patil, P. and B. M. C. Reddy (2008). Ex situ characterization and evaluation of pummelo

germplasm. *National Symposium on Citriculture: emerging trends.* NRCC, Nagpur, 24-27.

Paudyal, K. P. and Haq, N. (2008). Variation of pomelo (*Citrus grandis* (L.) Osbeck) in Nepal and participatory selection of strains for further improvement. *Agrofor. Syst.*, **72**: 195-204.

Paul, D. K. and Shaha, R. K. (2004). Citrus fruits in the northern region of Bangladesh. *Pakistan J. Biol. Sci*, **7**: 238-242.

Peach K and Tracey M.V. (1956) Modern methods of plant analysis, Vol I, Springer, Berlin G/Sttingen Heidelberg.

Pertuzatti, P. B.; Sganzerla, M.; Jacques, A. C.; Barcia, M. T. and Zambiazi, R. C. (2015). Carotenoids, tocopherols and ascorbic acid content in yellow passion fruit (*Passiflora edulis*) grown under different cultivation systems. *LWT-Food Sci. Technol.*, **64**(1): 259-263.

Pohl, H. R.; Wheeler, J. S. and Murray, H. E. (2013). Sodium and potassium in health and disease. *Met. ions Life sci.*, **13**: 29–47.

Pope, M. and Elder, J. (2023). Scurvy: An elusive diagnosis. *Clin. case rep. red*, **11**(6): e7418.

Powell, A. A. (1986). Cell membranes and seed leachate conductivity in relation to the quality of seed for sowing. *J. Seed Tech.*, **10**(2): 81-100.

Powell, A. A. (1989). The importance of genetically determined seed coat characteristics to seed quality in grain legumes. *Ann. Bot.*, **63**:169-195.

Pruthi, J.S.; Manan, J.K.; Teotia, M.S.; PodhakriShnasetty, G.; Epison, W.E.; Saroja, S. and K.C. Chikkappaji. (1984). Studies on the utilization of Kinnow and Malta. *J. Food Sci. Tech*., **21**(3): 123-127.

Rabha, A.; Wangchu, L. and Singh, B. (2013). Studies on genetic diversity of *Citrus* in east Siang district of Arunachal Pradesh. *Int. J. Agric. Environ. Biotechnol.*, **6**(1): 131-137.

Raghavan, M.; Singh, S. R.; Hazarika, B. N.; Wangchu, L. and Kumar, A. (2022). Genetic diversity of Mandarins from Northeast India along with three other wild species using morphological markers. *Pharma Innov. J.*, **11**(2): 1762-1771.

Rahman, M.M.; Rabbani, M.G.; Khan, A.; Ara, N. and Rahman M.O., (2003). Study on

Physio-morphological characteristics of different local Pummelo accessions. *Pak. J. of Biol. Sci.*, **6**(16): 1430-1434.

Rai, M.; Chandel, K.P.S. and Gupta, P.N. (1997). Occurrence, distribution, and diversity in the genus *Citrus* in the Indian gene centre. In: *Proceedings of the International Society of Citriculture*, 1228–1234.

Randazzo, W.; Jiménez-Belenguer, A.; Settanni, L.; Perdones, A.; Moschetti, M.; Palazzolo, E.; Guarrasi, V.; Vargas, M.; Germanà, M.A. and Moschetti, G. (2016). Antilisterial effect of *Citrus* essential oils and their performance inedible film formulations. *Food Control*, **59**: 750–758.

Ranganna, S. (1986). *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*. Tata McGraw-Hill Publishing Company Limited, New Delhi.

Raza, H.; Jaskani, M. J.; Khan, M. M. and Malik, T. A. (2003). In vitro induction of polyploids in watermelon and estimation based on DNA content. *Int. J. Agri. Biol*, **5**(3): 298-302.

Rees, A.; Dodd, G. F. and Spencer, J. P. (2018). The effects of flavonoids on cardiovascular health: A review of human intervention trials and implications for cerebrovascular function. *Nutrients*, **10**(12): 1852.

Roberto, D.; Micucci, P.; Sebastian, T.; Graciela, F. and Anesini, C. (2010). Antioxidant activity of limonene on normal murine lymphocytes: Relation to H_2O_2 modulation and cell proliferation. *Basic Clin. Pharmacol. Toxicol.*, **106**: 38–44.

Sacco, S.M.; Horcajada, M.N. and Offord, E. (2013). Phytonutrients for bone health during ageing. *Br. J. Clin. Pharmacol.*, **75**:697–707.

Sadeghnezhad, Z.; Amiri, S.; Rezazadeh-Bari, M. and Almasi, H. (2020). Physical and Morphological Characteristics of Edible Composite Film of Sodium Caseinate / Pectin / Zedo Gum Containing Poulk (*Stachys schtschegleevii*) Extract: Optimizing Bioactivity and Physicochemical Properties. *J. Pack. Technol. Res.*, **4**(2): 187–203.

Saeid, A. and Ahmed, M. (2021). Citrus Fruits: Nutritive Value and Value-Added Products. In *Citrus-Research, Development and Biotechnology*. IntechOpen. pp 1-18.

Saini, R. K.; Ranjit, A.; Sharma, K.; Prasad, P.; Shang, X.; Gowda, K. G. M. and Keum, Y. S. (2022). Bioactive compounds of *Citrus* fruits: A review of composition and health benefits of carotenoids, flavonoids, limonoids, and terpenes. *Antioxidants*, **11**(2), 239.

Sandhu, Kulwant, S.; Minhas, Kuldip S. and Sidhu, Jiwan S. (2012). Processing of citrus juice. In *Handbook of fruits and fruit processing.* Sinha, N. K., Sidhu, J., Barta, J., Wu, J., and Cano, M. P. (Eds.). John Wiley and Sons. pp 489-524.

Sarkar, M.; Gupta, N. and Assaad, M. (2019). Monitoring of fruit freshness using phase information in polarization reflectance spectroscopy. *Appl. Optics*, **58**(23): 6396-6405.

Sayed, H. A.; Hussein, S. A.; Ahmed, E. B.; Medeiros Filho, S. and Gallao, M. I. (2006). Morphological and physiochemical characterization of ten lime and lemon accessions and the assessment of their genetic diversity maintained at ISSR marker. *J. Hort. Sci. Orna. Pl.*, **8**: 200-211.

Scora, R. W. (1975). On the History and Origin of Citrus. *J. Torrey Bot.*, **102**(6): 369–375.

Scott, F. M. and Baker, K. C. (1947). Anatomy of Washington navel orange rind in relation to water spot. *Bot. Gaz.*, **108**(4): 459-475.

Seki, T.; Kamiya, T.; Furukawa, K.; Azumi, M.; Ishizuka, S.; Takayama, S.; Nagase, S.; Arai, H.; Yamakuni, T. and Yaegashi, N. (2013). Nobiletin-rich *Citrus reticulata* peels, a kampo medicine for Alzheimer's disease: a case series. *Geriatr. Gerontol. Int.*, **13**:236–238.

Seymour, G. B. (2002). Genetic identification and genomic organization of factors affecting fruit texture. *J. Exp. Bot.*, **53**(377): 2065–2071.

Sgroppo, S.C.; Pereyra, M.V.; Wlosek Stañgret, C.R. and Canteros, B.I. (2015). Characterization of Grapefruit (*Citrus paradisi*) Fruits Produced in Argentina and Paraguay. *Acta Hortic.*, **1065**: 267-271.

Sharifi-Rad, J.; Sureda Gomila, A.; Tenore, G.; Daglia, M.; Sharifi-Rad, M. and Valussi, M. (2017). Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. *Molecules*. **22**(1):70.

Sharma, B.D.; Hore, D.K. and Gupta, S.G. (2004). Genetic resources of *Citrus* of north-eastern India and their potential use. *Genet. Resour. Crop Evol.*, **51**: 411–418.

Sharma, Nimisha; Dubey, A.K.; Srivastav, M.; Singh, B.P.; Singh, A.K. and Singh, N.K. (2015). Assessment of genetic diversity in grapefruit (*Citrus paradisi* Macf) cultivars using physicochemical parameters and microsatellite markers. *Aust. J. Crop Sci.*, **9**(1): 62-68.

Shaw, P. E. (1979). Review of quantitative analyses of citrus essential oils. *J. Agric. Food Chem.*, **27**(2): 246–257.

Shen, W.; Xu, Y. and Lu, Y.H. (2012). Inhibitory effects of *Citrus* flavonoids on starch digestion and antihyperglycemic effects in HepG2 cells. *J. Agric. Food Chem.*, **60(**38**)**: 9609–9619.

Shravan, R. and Shere, D. M. (2018). Study of physico-chemical characteristics of sweet orange (*Citrus sinensis*) fruit. *J. Pharmacogn. Phytochem.*, **7**(6): 1687-1689.

Shrestha, N.; Shrestha, S. and Bhattarai, A. (2016). Determination of ascorbic acid in different *Citrus* fruits of Kathmandu Valley. *J. Med. Biol. Sci. Res.*, **2**(1): 9-14.

Singh, B (1981) *Establishment of first gene sanctuary for Citrus in Garo hills*. Concept Pub. Co., New Delhi, p 182

Singh, G.; Rattanpal, H.; Gupta, M and Sidhu, G. (2022). Genetic variability and heritability among mandarin (*Citrus reticulata* Blanco) genotypes under indian sub-tropical conditions. *Appl. Ecol. Environ. Res*. **20**:2913-2930.

Singh, H.; Rattanpal, H. S.; Sidhu, G. S. and Chahal, T. S. (2010). Study on physio-morphological characteristics among six rangpur lime (*Citrus limonia* Osbeck.) strains. *J. Tree Sci.*, **29**(1and2): 48-56.

Singh, I. P. (2004). Exploration, Collection and Characterization of *Citrus* Genetic Diversity from Manipur and Tripura. *Indian J. Plant Genet. Resour.*, **17**(2): 128-132.

Singh, I. P. and Singh, S. (2003). *Citrus* genetic diversity in central India. *Indian J. Plant Genet. Resour.*, **16**(3): 184-189.

Singh, I. P. and Singh, S. (2003). Exploration, collection, and mapping of *Citrus* genetic diversity in India. *Technical bulletin*, **7**.

Singh, I.P. and Govind, S. (1999). Variability in physico-chemical characteristics of indigenous citrus species/landraces of NEH region of India. In: *Hi-Tech Citrus Management Singh*, Shyam, S. and Ghosh, S.P. (Eds.)., ISC, NRCC, Nagpur, pp. 91-96.

Singh, I.P. and Singh, S. (2003). Exploration, collection and mapping of *Citrus* genetic diversity in India. Technical Bulletin No.7, National Research Centre for *Citrus*, Nagpur.

Singh, I.P. and Singh, S. (2004). Evaluation of seedless cultivars of mandarin (*Citrus reticulata*) for export. *Ind. J. Agric. Sci.*, **74**(7): 376-378.

Singh, I.P. and Singh, S. (2006). *Citrus* monograph, NRCC, Nagpur, India. pp. 1-96.

Singh, N. P.; Gill, P. S.; Jawandha, S. K. and Kaur, H. (2009). Genetic Variability in Hill Lemon Strains (*Citrus pseudolimon* Tanaka) under Punjab Conditions. *Not. Bot. Horti Agrobot. Cluj Napoca,* **37**(1): 238-243.

Smirnoff N. (2018) Ascorbic acid metabolism and functions: A comparison of plants and mammals. *Free Radic. Biol. Med.* **122**:116–129.

Stewart I. (1975). Influence of tree position of citrus fruit on their peel and juice color. *Proc. Florida St. Hort. Soc*., **88**: 312–314.

Suri, S.; Singh, A.; Nema, P. K.; Malakar, S. and Arora, V. K. (2022). Sweet lime (*Citrus limetta*) peel waste drying approaches and effect on quality attributes, phytochemical and functional properties. *Food Biosci.,* **48**: 101789.

Suwannayod, S.; Sukontason, K.L.; Somboon, P.; Junkum, A.; Leksomboon, R.; Chaiwong, T.; Jones, M.K.; Sripa, B.; Balthaisong, S. and Phuyao, C. (2018). Activity of kaffir lime (*Citrus hystrix*) essential oil against blow flies and housefly. *Southeast Asian J. Trop. Med. Public Health,* **49**: 32–45.

Svoboda, K. P. and Greenaway, R. I. (2003). Lemon-scented plants. *International J. Aromatherapy*, **13**(1): 23-32.

Swingle W.T. and Reece P.C. (1967). The botany of *Citrus* and its wild relatives. In: *The Citrus industry*, Reuther W., Webber H.J., Batchelor L.D. (Eds). University of California, Berkeley, **1**: 190–430.

Tabatabaeefar, A. and Rajabipour, A. (2005). Modelling the mass of apples by geometrical attributes. Sci. Hortic., **105**: 373-382.

Tadeo, F. R.; Cercós, M.; Colmenero-Flores, J. M.; Iglesias, D. J.; Naranjo, M. A.; Ríos, G. and Talon, M. (2008). Molecular physiology of development and quality of citrus. *Adv. Bot. Res.,* **47***:* 147-223.

Tanaka T. (1937). Further revision of Rutaceae-Auranticeae of India and Ceylon. *Ind. Bot. Soc.,* **16**: 227-240.

Tanaka, T. (1928). On certain new species of *Citrus*. *Stud. Citrol.,* **2**: 155-164.

Tanaka, T. (1928). Revisio Aurantiacearum-I. *Bull. Soc. Bot. France*, **75**(4): 708-715.

Tanaka, T. (1937). Further revision of Rutaceae-Aurantioideae of India and Ceylon. (Revision aurantiacearum VIII.) *J. Indian. Bot. Soc*., **16**: 227–240.

Tanaka, T. (1959). Origin of *Citrus* fruits with reference to Himalaya region. *Hortic. Soc. J.*, **28**(2): 71-75.

Tao, N. G.; Liu, Y. J. and Zhang, M. L. (2009). Chemical composition and antimicrobial activities of essential oil from the peel of bingtang sweet orange (*Citrus sinensis* Osbeck). *Int. J. Food Sci. Technol.*, **44**(7): 1281-1285.

The Observatory of Economic Complexity. (2019). Economic Complexity Ranking. https://oec.world/en/profile/hs/citrus.html retrieved on 24 March 2023

Thi Minh Tu, N.; Sawamura, M.; Onishi, Y.; Ogawa, E. and Choi, H. S. (2004). Characteristic odour components of *Citrus reticulata* Blanco (Ponkan) cold-pressed oil. *Biosci. Biotechnol. Biochem.*, **68**(8): 1690-1697.

Thimmaiah, S. K. (1999). Standard methods of biochemical analysis. Kalyani Publishers, New Delhi, India.

Ting, S. V. and Attaway, J. A. (1971). Citrus fruits. In: *The biochemistry of fruits and their products*. Hulme, A. C., (Ed.)., Academic Press, London, pp 107-169.

Tisserat, B.; Galletta, P. and Jones, D. (1990). Carpel Polymorphism in Citrus Fruit. *Bot. Gaz.*, **151**(1): 54–63.

Tolkowsky, S. (1938). *Hesperides. A history of the culture and use of Citrus fruits.* J Bale, Sons and Curnow Ltd., London

Tripodo, M. M.; Lanuzza, F.; Micali, G.; Coppolino, R. and Nucita, F. (2004). *Citrus* waste recovery: a new environmentally friendly procedure to obtain animal feed. *Bioresour. Technol.*, **91**(2): 111-115.

Trong, L and Thinh, B. (2018). Study of nutritional ingredients of oranges (*Citrus sinensis (L.) Osb.*) grown in Vietnam. *J. Agric. For. Fish.*, **4:** 20-25.

Tu, M.N.T.; Onishi, Y.; Choi, H.S.; Kondo, Y.; Bassore, S.M.; Ukeda, H. and Sawamura, M. (2002). Characteristic odour components of *Citrus sphaerocarpa* Tanaka (Kabosu) cold-pressed peel oil. *J. Agric. Food Chem.*, **50**: 2908-2913.

Tyl, C. and Sadler, G. D. (2017). pH and titratable acidity. In: *Food analysis*, Neilsen, S.S (Ed.). Food Science text series, Springer, Boston, pp 389-406.

Uckoo, R. M.; Jayaprakasha, G. K.; Nelson, S. D. and Patil, B. S. (2011). Rapid simultaneous determination of amines and organic acids in *Citrus* using high-performance liquid chromatography. *Talanta*, **83**(3): 948-954.

Valacchi, G.; Sticozzi, C.; Belmonte, G.; Cervellati, F.; Demaude, J. and Chen, N. (2015). Vitamin C compound mixtures prevent ozone-induced oxidative damage in human keratinocytes as initial assessment of pollution protection. *PLoS One,* **10**(8): e0131097.

Vavilov, N. I. (1927). Geographical regularities in the distribution of the genes of cultivated plants. *Bull. Appl. Bot.*, **17**(3): 411-428.

Verma, S.; Bhatnagar, P. and Yadav, A. (2012). Physico-chemical, yield and yield attributing characteristics of Nagpur mandarin (*Citrus reticulata* Blanco) orchards surveyed in Jhalawar district of Rajasthan. *Asian J. Hort.*, **7**(2): 437-441.

Violeta, N.; Trandafir, I. and Ionica, M. E. (2010). HPLC organic acid analysis in different *Citrus* juices under reversed phase conditions. *Not. Bot. Horti Agrobot. Cluj Napoca*, **38**(1): 44-48.

Voragen, A. G. J. and Van den Broek, L. A. M. (1991). Fruit juices. In: *Biotechnological Innovations in Food Processing*. J. Green (Ed.)., Butterworth-Heinemann Ltd, Oxford, pp. 187-210.

War, A.R.; Paulraj, M.G.; Ahmad, T.; Buhroo, A.A.; Hussain, B.; Ignacimuthu, S. and Sharma, H.C. (2012) Mechanisms of plant defense against insect herbivores. *Plant Signal. Behav.,***7:** 1306–1320.

Wazed, M. A.; Hasan, M.; Halim, M. A.; Akhtaruzzaman, M.; Ahmed, M. and Mozumder, N. R. (2022). Quantitative analysis of vitamins B_2, B_3, B_5 and B_6 by HPLC-UV in selected non-leafy vegetables. *Int. J. Veg. Sci.*, **28**(5): 493-499.

Webber, H.J. (1967). History and development of the Citrus industry. In: *The Citrus Industry Vol. 1*, Reuther, W.; Webber, H.J. and Batchelor, L.D. (Eds.). University of California Press, Berkeley: 1-39.

Whitaker, J.R.(1984). Pectic substances, pectic enzymes and haze formation in fruit juices. *Enzyme Microb.*, **6**: 341-349.

Wiegand, C.L.; Swanson, W.A. and Cruse, R.R. (1982). Marrs, Valencia and Ruby Red juice quality as affected by irrigation plus rainfall. *J. Rio Grande Valley Hort. Soc,*. **35**: 109–120.

Wrolstad, R.E. (1981). Use of sugar, sorbitol, and nonvolatile acid profile in determining the authenticity of fruit juice concentrates. In: *Proceedings of the symposium on technological problems of fruit juice concentrates*. Oregon State University:

Corvallis, pp. 27- 39

Wu, G.A.; Terol, J.; Ibanez, V.; López-García, A.; Pérez-Román, E.; Borredá, C.; Domingo, C.; Tadeo, F.R.; Carbonell-Caballero, J.; Alonso, R. and Curk, F. (2018) Genomics of the origin and evolution of *Citrus*. *Nature,* **554**: 311–316.

Xie, J.; Wang, G.; Ren, T.; Li, J.; Chen, S.; Shi, L. and Liu, H. (2023). The gradient distribution of pigment metabolites provided insights into the uneven colouration of pulp in cold-stored blood orange. *Postharvest Biol. Technol.,* **198**: 112234.

Xu, G.; Liu, D.; Chen, J.; Ye, X.; Ma, Y. and Shi, J. (2008). Juice components and antioxidant capacity of *Citrus* varieties cultivated in China. *Food chem.,* **106**(2): 545-551.

Xu, J.; Tao, N.; Liu, Q. and Deng, X. (2006). Presence of diverse ratios of lycopene, carotene in five pink or red-fleshed citrus cultivars. *Scientia. Horticulturae*. **108:** 181-184.

Yamaki, Y. T. (1989). Organic acids in the juice of *Citrus* fruits. *J. Jpn. Soc. Hortic. Sci.*, **58**(3): 587-594.

Zaidun, N. H.; Thent, Z. C. and Abd Latiff, A. (2018). Combating oxidative stress disorders with *Citrus* flavonoid: Naringenin. *Life sci.*, **208**: 111-122.

Zekri, M. (2011). Factors affecting citrus production and quality. *Trees*. https://crec.ifas.ufl.edu/media/crecifasufledu/extension/extension-publications/2011/2011_Dec_factors_citrus.pdf retrieved on 24 April, 2023

Zekri, M. (2011). Factors affecting citrus production and quality. *Trees*

Zhang, H.; Xie, Y.; Liu, C.; Chen, S.; Hu, S.; Xie, Z. and Xu, J. (2017). Comprehensive comparative analysis of volatile compounds in citrus fruits of different species. *Food chem.,* **230:** 316-326.

Zhang, J. and Ritenour, M. A. (2016). Sugar composition analysis of commercial *Citrus* juice products. *Proc annu. meet Fla.,* **129**: 178-180.

Zhang, W.; Liu, D.; Fu, X.; Xiong, C. and Nie, Q. (2022). Peel Essential Oil Composition and Antibacterial Activities of *Citrus x sinensis* L. Osbeck ‘Tarocco’and *Citrus reticulata* Blanco. *Horticulturae*, **8**(9): 793.

Zohra, H. F.; Rachida, A.; Malika, M.; Benali, S.; Samir, A. A. and Meriem, B. (2015). Chemical composition and antifungal activity of essential oils of Algerian *Citrus*. *Afr. J. Biotechnol.,* **14**(12): 1048-1055.

Zou, Z.; Xi, W.; Hu, Y.; Nie, C. and Zhou, Z. (2016). Antioxidant activity of *Citrus* fruits. *Food chem.*, **196**: 885-896.

Appendices… ✍

APPENDIX I

UHPLC CHROMATROGRAMS OF ORGANIC ACID SAMPLES

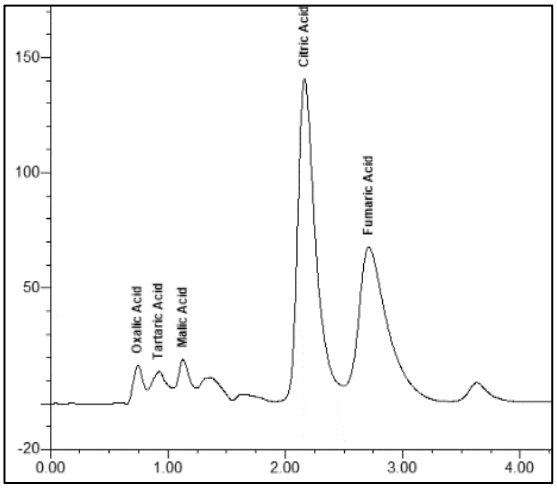


Fig 1: Rangpur lime

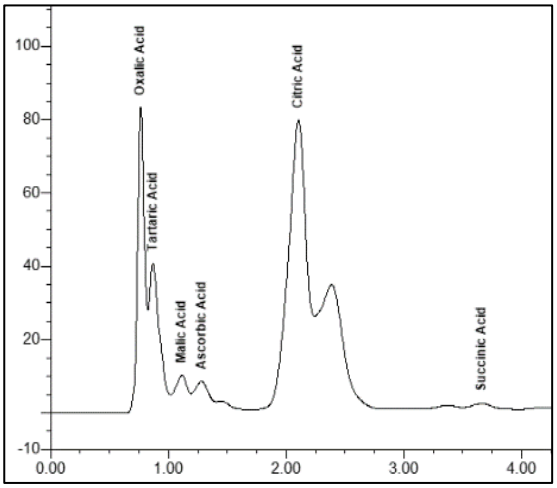


Fig 2: Bira jora

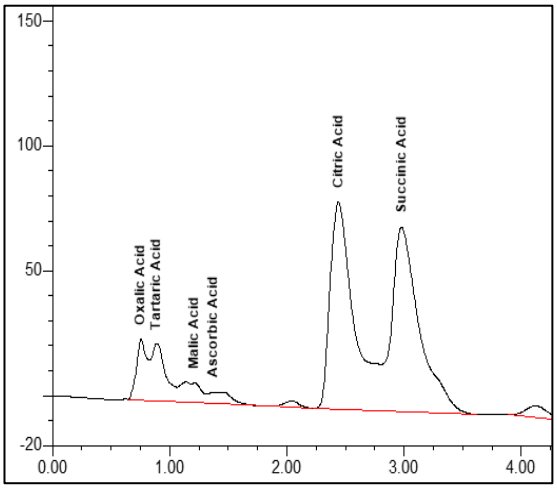


Fig 3: Sour orange

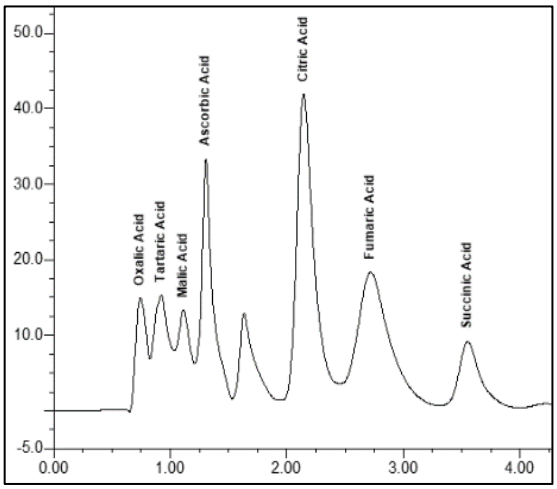


Fig 4: Sweet orange

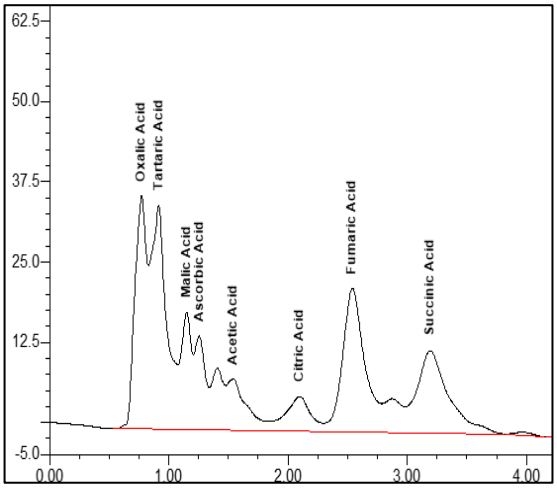


Fig 5: Khasi mandarin

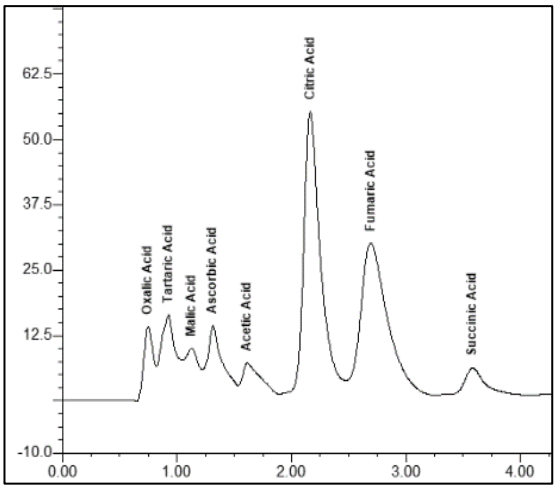


Fig 6: Cleopatra mandarin

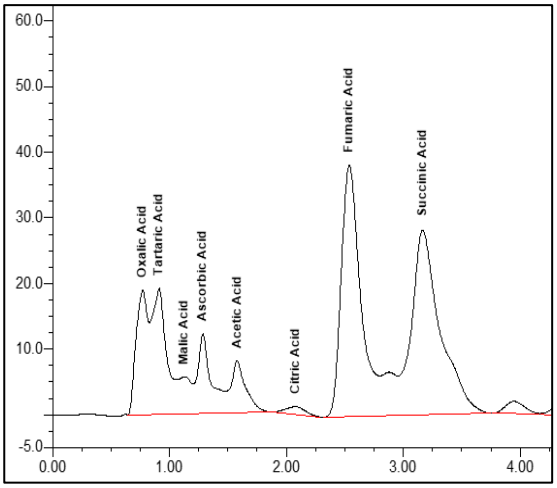


Fig 7: Pummelo

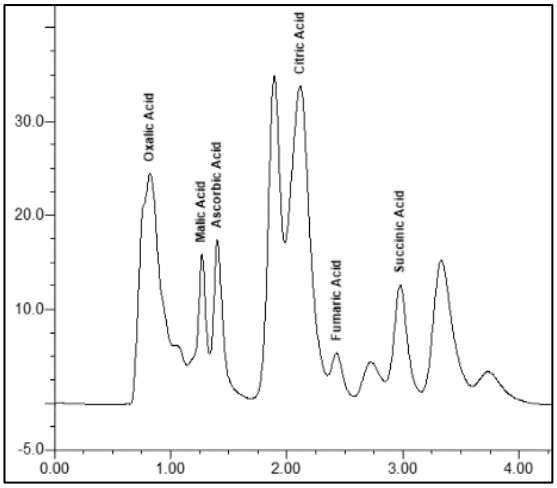


Fig 8: Grapefruit

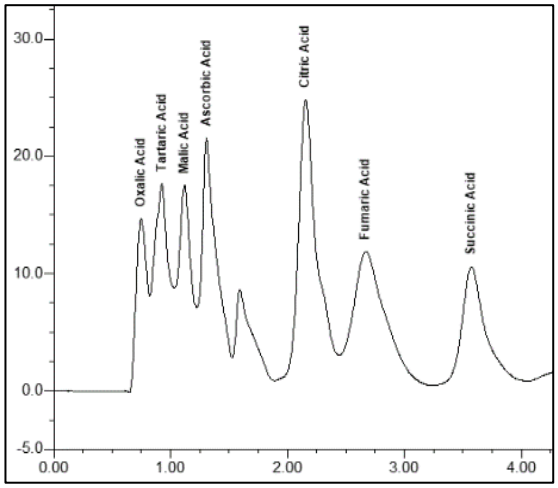


Fig 9: Kaffir lime

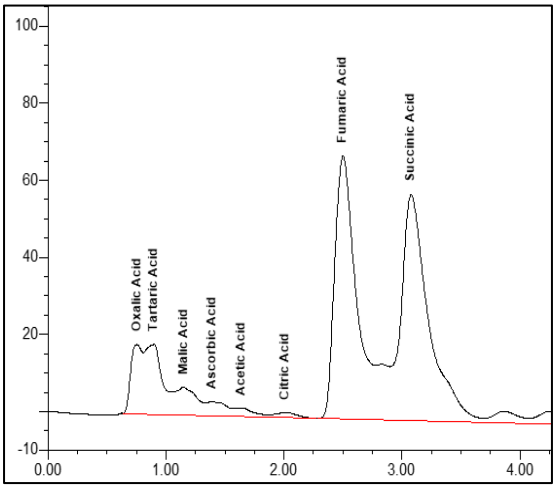


Fig 10: Khasi papeda

APPENDIX II

UHPLC CHROMATROGRAMS OF WATER- SOLUBLE VITAMIN SAMPLES

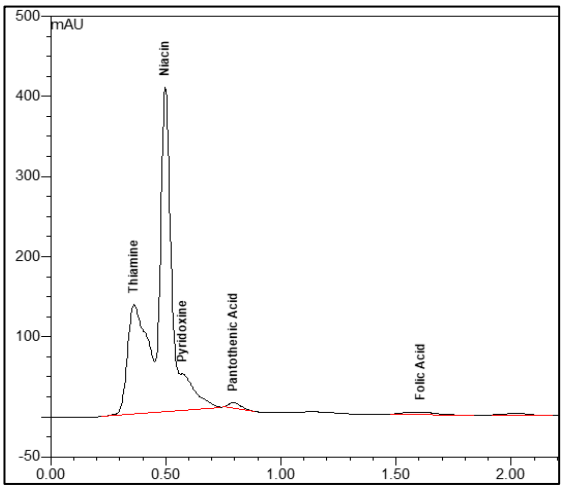


Fig 1: Rangpur lime

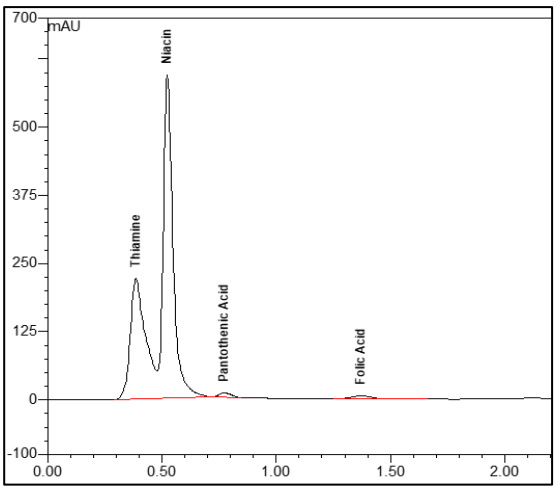


Fig 2: Bira jora

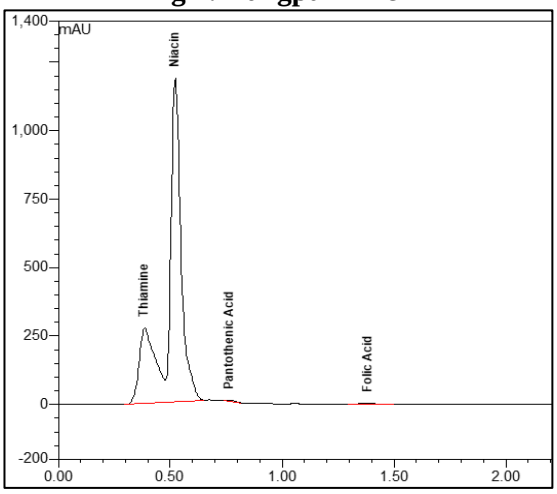


Fig 3: Sour orange

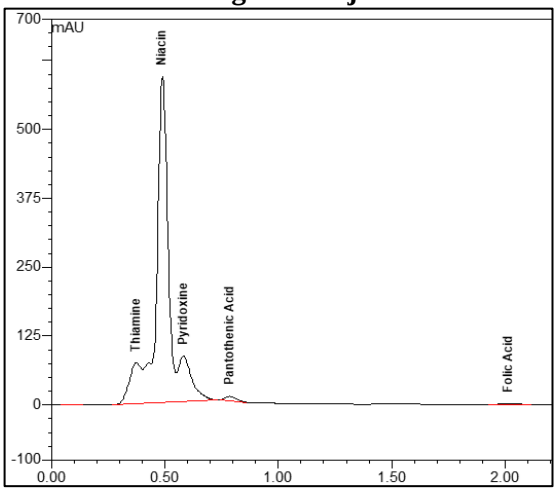


Fig 4: Sweet orange

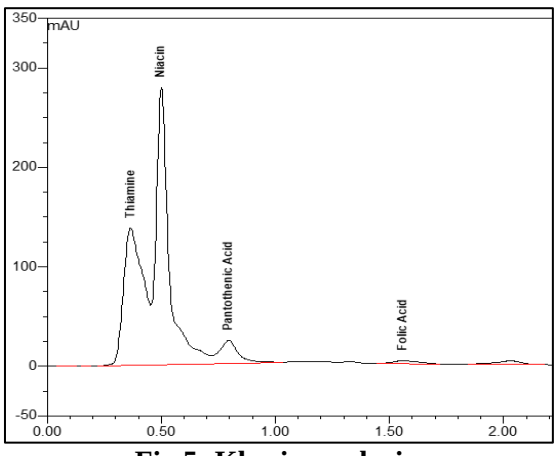


Fig 5: Khasi mandarin

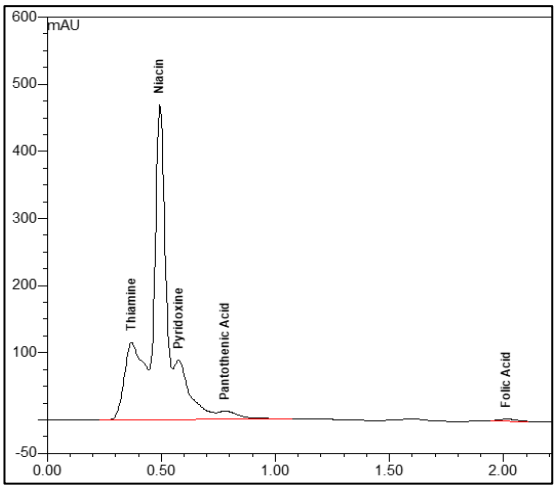


Fig 6: Cleopatra mandarin

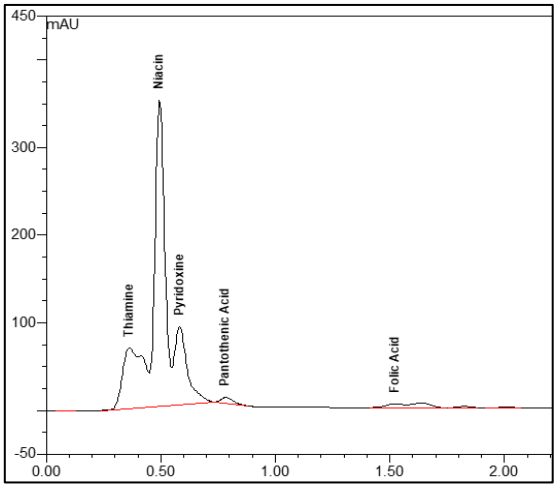


Fig 7: Pummelo

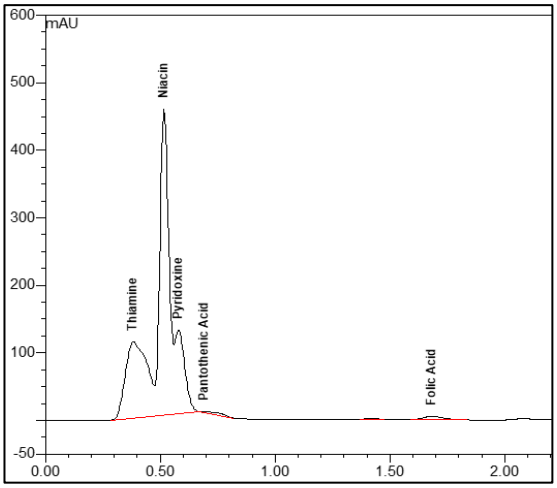


Fig 8: Grapefruit

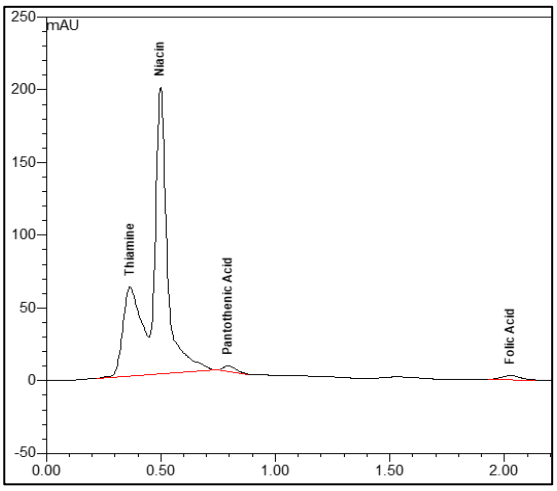


Fig 9: Kaffir lime

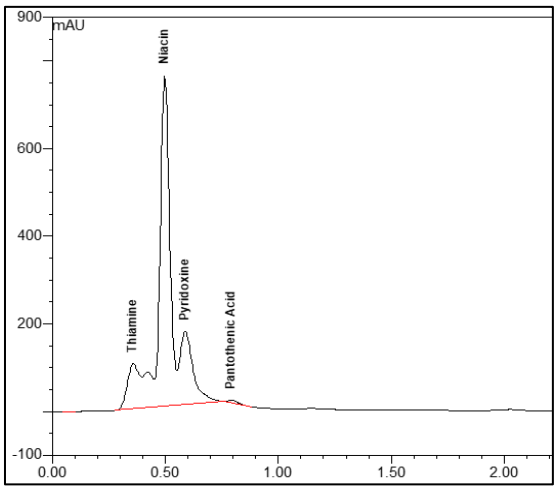


Fig 10: Khasi papeda