ESSENTIAL OIL, PECTIN AND IONOME PROFILING OF CITRUS FRUIT WASTE

A Thesis Submitted

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Contents

Abbreviations

MSL	Mean Sea Level
FAO	Food and Agriculture Organization
DM	Dry Matter
GAE	Gallic acid equivalent
ND	Non detectable
FW	Fresh weight
DPPH	2,2- Diphenyl-1-1- Picrylhydrazyl Assay
DDW	Doubled distilled water
G	Gram
mg	Milligram
kg	Kilogram
%	Percentage
°C	Degree Centigrde
° B	Degree Brix
µg	Microgram
µl	Micro litre
ppm	parts per million
TSS	Total Soluble Solid
FSSAI	Food Safety and Standards Authority of India
GCMS	Gas Chromatography Mass Spectrometry
GPS	Global Positioning System
ICPMS	Inductively Coupled Plasma Mass Spectrometry
FCC	Food Chemical Codex

HMP	High methoxyl pectins
LMP	Low methoxyl pectins
IOC	International Olive Council
AUA	Anhydrounic acid
ATP	Adenosine tri phosphate
MeO	Methoxyl Content
RDA	Recommended dietary allowance
DE	Degree of Esterification
FFA	Free fatty acid
QE	Quercetin
TA	Titrable acidity
NA	Not available
RI	Refractive Index
IV	Iodine Value
TV	Thiocyanogen value
FTIR	Fourier Transformation Infrared Spectroscopy
Eq. Wt.	Equivalent Weight
DAF	Days after fruit set
mm	Milimetre
mM	Milimole
nm	Nanometer
ml	Mililitre
L	Litre
µg	Micro gram
Ca	Calcium

Mg	Magnesium
P	Phosphorus
Zn	Zinc
Na	Sodium
K	Potassium
N	Nitrogen
Cu	Copper
Cd	Cadmium
Pb	Lead
Se	Selenium
Mn	Manganese
Ni	Nickel
Co	Cobalt
Ce	Cerium
Mo	Molybdenium
Fe	Iron
I	Iodine
Hg	Mercury
KOH	Potassium Hydroxide
Al	Aluminium
Ag	Silver
Ba	Barium
Si	Silicon
B	Boron
Li	Lithium

Cs	Caesium
S	Sulphur
m.e.	milli equivalent
QE	Querecetin extract
GAE	Gallic acid extract
RI	Refractive Index
SV	Saponification value
IV	Iodine value
FFA	Free fatty acid
TV	Thiocyanogen value
CD	Critical Difference

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Chapter 1

Introduction

Citrus fruit is widely distributed throughout the subtropical and tropical regions of Asia and the Malay Archipelago and believed to be originated in Southeast Asia, regions extending from North-East India eastward through the Malayan Archipelago (Swingle and Reece,1967; Moore,2001). The oldest known reference of citrus appeared before 800 BC in Sanskrit literature and is now found growing over 100 countries geographically extending from 40° North to 40° South Equator with annual production of more than 64 million tonnes of fruits (Subba, 2014).

Citrus fruit ranks first in terms of area and production in the world followed by banana and grapes (Horticultural Statistics at a glance, 2018). Being grown in more than 100 countries in various climatic condition *viz.* tropical, subtropical and mediterranean it is taken as the most remunerative fruit crops in the international trade and world scenario.

The major commercial citrus fruits are sweet orange (*Citrus sinensis* (L), tangerines/mandarins (*Citrus reticulata* Blanco), lemon (*Citrus limon* Burm.), lime (*Citrus aurantifolia* Swingle), grapefruit (*Citrus paradisi* Macf), pommelo (*Citrus grandis* Osb.) and other hybrids. The world citrus area was 9.45 ha with production was 146.43 million tones. China is the highest producer of citrus with 38.39 million tonnes followed by others with 37.31 million tonnes and Brazil with 19.59 million tons (Horticultural Statistics at a glance, 2018).

The production of citrus in world is dominated by sweet orange contributing 64 % of the production followed by mandarin (20 %), limes and lemon (10 %), grape fruit (6 %) and other. whereas mandarin alone occupies more than 55 % of total area among other citrus fruits in India.

Citrus occupies 3rd position in India next to mango and banana cultivated in terms of production and 2nd postion with regards to area after mango with an area of 100 thousand hectare with the production of 12546 thousand metric tonnes. Amongst citrus fruits, mandarin group of citrus are cultivated in 428 thousand hectares with the production of 5101 thousand metric tonnes. Limes are cultivated in an area of 286 thousand hectare with production of 3148 thousand metric tonnes. Sweet orange covers the considerable area of 103 thousand hectare with production of 1030 thousand metric tonnes (Horticultural Statistics at a Glance, 2018)

The species commercially cultivated in India are *Citrus sinensis* Osbeck (sweet orange), *Citrus reticulata* Blanco (mandarin), *Citrus aurantifolia* Swingle (acid lime) and *Citrus limon* (L) Burm f. (lemon). There are several cultivars of these species which are grown in different agro-ecological conditions of this country (Subba, 2014).

Northeastern region is the citrus depository in India where many citrus species are originated. Khasi mandarin (*Citrus reticulata*) is widely cultivated in Northeast India and Sweet orange (*Citrus sinensis*) is also commercially grown in some of the places in the region. Apart from the most commonly cultivated species *Citrus indica* Tanaka (Indian wild orange), *Citrus latipes* (Swingle), *Citrus ichangensis* Swingle (Ichang Papeda), *Citrus medica*, *Citrus assamensis*, *Citrus macroptera* and *Citrus hystrix* were reported to occur in the subtropical forests of North-East India and the foot hills of the East Himalayas (Malik *et al*., 2006). North East region of India is home for many *Citrus species* have a diversity of 23 taxa including 68 varieties (Sharma *et al*., 2004).

A tiny Himalayan state in North East India, Sikkim harbors many citrus species and *Citrus reticulata* is most commercial. Other species of citrus found growing in the states are rough lemon-kali jyamir *(Citrus jambhiri).* Sikkim mandarin is found growing commercially following organic package of practices at the altitude range of 300 m-1800 m from sea level (Yadav *et al.,* 2016). Sikkim mandarin is cultivated in an area of 13080 ha in Sikkim with production of 18,999 MT (Horticulture statistics at a glance, 2018).

Citrus fruits have its own characteristics, usually have rough, robust and bright (green to yellow) coloured skin. They are usually 4 to 30 cm long and 4 to 20 cm in diameter, with a leathery surrounding rind or skin known as epicarp (or flavedo) that cover the fruits and protect it from damages. Citrus fruits are notable for their fragrance, partly due to flavonoids and limonoids present in the rind (Manthey, 2004). The endocarp is rich in soluble sugar and contains significant amounts of vitamin C, pectin, fibres, different organic acids and potassium salt which give the fruits its characteristic citrus flavour (Ezejiofor *et al*., 2011).

Citrus fruit is fat, sodium and cholesterol free which are associated with cardio vascular diseases. They have substantial quantities of essential mineral nutrients like potassium, calcium, phosphorus, magnesium, copper and vitamins like thiamine, folate which also help to reduce heart diseases and types of cancer. Citrus fruits are good source of vitamin C, citric acid, flavonoids, phenolics, pectins, limonoids, etc. One or other type of citrus fruits is always available throughout the year.

Citrus fruit after its utilization as table fruit or processed for juice production generates huge waste in the form of peel and pomace which is half of the whole fruit mass (Cohn and Cohn 1977, Braddock,1999). Large volume of solid and liquid wastes generated from the food industry can be used as raw material for pectin extraction or used (without treatment) for the production of animal feed or fertilizers (Mamma *et al*., 2008).

The peels of citrus fruits are well studied as a source of pectin. Pectin is a structural hetero polysaccharide and finds commercial use as a gelling agent and stabilizer in food industries. It is a complex mixture of polysaccharides occurring in the primary cell walls of terrestrial plants and is a high value functional food ingredient (William *et al*., 2005). Pectin is produced commercially in the form of white to light brown powder, mainly extracted from citrus fruits and is used in food as a gelling agent particularly in jams and jellies. It is also used in filling sweets, as a stabilizer in fruit juices and milk drinks and as a source of dietary fibre (Tobias *et al*., 2011).

Essential oil from citrus peel imparts their own flavours as well as fragrances which can be used not only in various food industries but also in day to day using cosmetics and health related products (Sheng-min *et al*., 2012). Citrus species are a potential source of volatile oil which might be utilized for edible and other industrial application. Essential oils are broadly used as pharmaceutical components, in nutritious supplements and for cosmetic industry and aromatherapy (Colecio-Juarez *et al.,* 2012). The oil is also employed in perfumes, toilet waters, fragrance and in cosmetics. These oils have potent antimicrobial components, having wide range of therapeutic constituents; they are often used for their flavour and their therapeutic properties, in a wide range of products including foods, medicines, and cosmetics (Ali, 2015).

Ionome study and nutrient signature is of paramount importance, if the plant products are used as food. The citrus peel and pomace after being processed can be utilized as animal feed. The study usually involves the quantitative and simultaneous measurement of the elemental composition of living organisms and changes in this composition in response to environmental, physiological stimuli, developmental state, and genetic modifications. Ionome analysis require the application of high-throughput elemental analysis technologies and their integration with both bio-informatic and genetic tools. The quantitative and simultaneous measurement of the elemental composition of living organisms requires choosing specialized instrumentation and sample preparation protocols.

Inductively coupled plasma (ICP) spectroscopy is most recent technique used for ionome analysis (Li *et al.,* 2013). The goal of ICP is to ionize atoms for their detection by either optical emission spectroscopy (ICP-OES) [also known as atomic emission spectroscopy (ICP-AES)] or mass spectrometry (ICP-MS). The advantage of ICP-MS is that it allows for a smaller sample size and has an edge in sensitivity and the ability to detect different isotopes of the same element.

Mineral nutrients and Heavy metals have their specific role in human body or they serve as cofactor for many physiologic and metabolic functions. Having thought that, the citrus peels may also serve as source of valuable nutrients required for normal functioning of the body system. The utilization of these peels will enhance conversion of waste to wealth. It will also contribute positively towards solid waste management and a cleaner environment (Ozcan *et al*., 2016).

The essential metals can produce toxic effects when taken in high concentrations, whereas non-essential metals are toxic even in very low concentration for human health. So, elemental contents of the plants need to be screened for their quality control. Recently, many studies worldwide reported the importance of elemental constituents of the herbal drug plants which enhanced the awareness about trace elements in these plants. The researchers are trying to link the contents of the trace elements and the medicinal values of the plants. Though much is known about the functional role of a number of elements, the best foreseeable benefit for human health, by mineral nutrition, lies in obtaining the correct amount of supplementation in the right form at the right time.

Despite accountable studies about essential oil and pectin in citrus fruit waste, there exist huge scope in understanding the essential oil and pectin content as a function of different geographical area with different farming system. Further the aim of this study is to study the major, minor and trace elemental levels in citrus fruit peel and pomace.

The biochemical composition like essential oil, pectin and elemental/ionomic of plants/ plant part(s) is greatly depends on the genetic variations and associated with climatic conditions of the geographical area where they grow. In the hills, there is change in the climatic parameters due to abrupt change in the altitude. It is obvious that changing altitudinal gradient may brought about changes in the above parameters. None of the citrus species of the Himalayan area of North East India, particularly, Sikkim have been subjected to study keeping the altitudinal gradient as major variables to bridge the research gaps as understood from the forgoing text. Present investigation was envisaged with the following objectives:

Objectives:

- Proximate analysis of peel and pomace of *Citrus* germplasms.
- Physico-chemical characterization of essential oil from peel of different *Citrus* germplasms.
- Estimation of pectin content in fruit peel and physico-chemical analysis of pectin.
- Ionome profiling of peel and pomace of *Citrus* germplasms.

Chapter 2

Review of Literature

The relevant available literaturepertaining to the scientific investigation had been undertaken. The review of the several works related to present investigation is being done to assist the delineation of objectives, hypothesis and research procedure to be followed. Citrus fruit peel and pomace left after extraction of juice are usually the waste, which create nuisance to the environment the disposal of waste either presents an added cost to processors or its direct disposal in soil or as garbage not leads to pollution in the environment. Therefore, utilization of peel and pomace is most sought proposition for additional income and to reduce the environmental hazards, which is a primary need for any new food processing industry. Extraction of oil, pectin and ionome profiling are the major strategies that can be thought for utilization of these wastes. The details of the work done and the related literature are being divided into different sub headings and have been summarized in tabular forms.

2.1 Waste management

Food waste has posed the serious challenges to food security. Lipinski, 2012 mentioned that 1314 trillion kcal per year can be saved by reducing 50 % current Food waste which is equivalent to 22 % of calories needed to feed the projected population by 2050. Reduction on food waste contributes for increasing economic productivity and reduction in emission of green house gases, major contributor for climate change (WRAP, 2015).

In food processing industry there is a huge waste being thrown after getting desired products. The pomace, peel and seed contribute to about 25-30 % of waste produced from processing of fruits and vegetables in the processing industry

(Galanakis, 2012). There have been several findings of research for further utilization of these wastes as nutritional products. Transformation of such waste to food value can have positive implication towards sustainable future.

2.2. Phenolic compounds

Citrus fruit waste particularly the peel is rich in natural flavonoids and contain higher amount of phenolics compounds. The total phenolics were found to be 15 % higher in peels of lemons, oranges, and grapefruit than those in the peeled fruits. Likewise, flavonoids in citrus are a major class of secondary metabolites. Peels contain highest amount of flavonoids than other parts.

The phenolic content of plant may be dependent on several factors such as geographical origin, clones, plant height, solvent, maturation stages, vegetative rootstock and extraction time (Sharma *et al*., 2001; Abeysinghe *et al*.,2007). Flavonoid variation was found due to genetic origin, the time of fruit collection, and the different parts of the used fruit (peel, and edible parts) (Lu *et al*., 2006). Further fruit development stage also affects the flavonoids content (Castillo *et al*.,1992; Ortuno *et al*., 1997). There are several beneficial aspects of flavonoid in citrus such as anticancer, antiviral, and anti□inflammatory activities and restrict human platelet aggregation (Benavente□Garcia *et al*., 1997). In addition, Zhang *et al*., 2012 mentioned consumption of two flavonoids; naringin and neohesperidin led to reduction of blood sugar and lipid profile level in human due to consumption of citrus fruit.

The peels of citrus are rich in phenolic compounds which can be extracted and use as natural antioxidants and can be used in functional foods (Patil *et al*., 2009; Albishi *et al*., 2013). Citrus fruits are rich source of antioxidants like ascorbic acid, flavonoids, phenolic compounds and pectins. Citrus by products are also found to be rich in naturally occurring flavonoids (Chanalia *et al*., 2018).

The citrus peels, pomace and seeds are also found to be rich in poly-phenols and is found to be twice that in edible tissue. It has anti-cancer, anti-microbial, anti-oxidative and immune-stimulating effects which reduces the incidence of several cardiovascular diseases (Wadhwa and Bakshi, 2013).

2.3. Antioxidants

The low concentration of antioxidant delays or prevents oxidation of that particular substance as compared to oxidisable substrate (Halliwell and Guteridge,1989). Vitamin C reduces racial from variety of sources and is a water-soluble antioxidant and as per Coinu *et al*., 2007 it acts as a proxidant under certain situation and produces oxygen by products of the metabolism which can cause damage to cells. Further phenolic compound is also attributed to antioxidants.

DPPH is a free radical scavenging activity with absorption at 517 nm which is found to decrease with scavenging of proton radical as illustrated by (Yamaguchi *et al.,*1998). Further, the decrease in DPPH is attributed to reaction between antioxidant molecule and radical which ultimately led to scavenging of the radical by donation of hydrogen (Hong and Chi-Tang, 1997)

In case of IC_{50}**,** a lower absorbance is related to higher scavenging effect. As mentioned by (Soares *et al*., 1997), antioxidant reacts with the DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and get converted to 1,1 diphenyl -2- picryl hydrazine, due to its hydrogen donating ability at a very rapid rate with de colouration. Compound having high antioxidant activity result in a rapid decline the DPPH absorbance (Guimaraes *et al*., 2010).

2.4. Proximate analysis

There is huge waste liberated by citrus processing industry every year and citrus peel alone contributes to about 50% of the wet mass in republic of Korea (Sharma *et al*., 2017). It has economic value as it contains abundant amount of flavonoids, carotenoids, dietary fiber, sugars, polyphenols, essential oils, ascorbic acid and considerable amounts of some trace element. Its application is also in bio ethanol production.

The citrus waste from industry is estimated to be around 40 million tons worldwide and the amount of residue obtained from the fruits accounts for ~50 % of the original whole fruit production (Marin *et al*., 2007).

As reported by (Crawshaw, 2004; Bakshi and Wadhwa, 2013) the pomace waste contains 5−10 percent crude protein and 6.2 percent ether extracts, 10-40 percent soluble fibre (pectin's) and 54 percent water soluble sugars .The high fibre content makes it a safer feed than cereals for making low-roughage diets as in high-yielding dairy cows (Crawshaw, 2004). As per Fegeros *et al.,* 1995, dried citrus pulp can replace 20 percent concentrate in dairy cattle and up to 30 percent in lactating ewes without affecting the dry matter intake, rumen metabolites and milk yield or milk protein and fat content.

The dietary fibre from citrus exhibited good quality than other sources of fibre because of presence of flavonoids, polyphenols, and carotene with antioxidant activities (Marin *et al*., 2007)

Table 2.1: Review on Proximate analysis of peel and pomace of different Citrus germplasms

Authors	Fruit	Place	Parameters reported
Adeline *et al*.,2016	Bitter orange peel	Chennai, India	Phenol (mg GAE/g):1.76,1.46,1.44; flavonoid (mg QE/g): 0.32, 0.036,0.086
	Lemon peel in fresh, microwave, air oven drying methods		Phenol mg/100g:1353.88,1321.31 ,1180.78; DPPH (%): (79.37, 56.69); (50.93,65.56); (56.01and 52.64)
Adewole *et al*., 2014	Unripe orange peel	Nigeria	Moisture:10.30%, fat (%):2.78, Crude fibre (%) :12.47; Protein (%):16.51
Ahmed Abd El-ghfar *et al*., 2016	Orange peel in fresh (control), microwave and air oven drying in methanol and ethanol extract	Egypt	Moisture(%): (74.35, 8.51, 9.96); protein (%): (7.09, 6.44, 6.39); fibre (%):(11.50, 10.40, 10.50);Ash (%): 4.21, 3.33, 3.51;total phenol (mg GAE/100g):2619.39, 1535.94, 1410.73 and 5255.02, 3026.34, 2453.75 ; DPPH (%): 99.79, 69.83, 56.29 and 98.76, 68.85, 53.83; flavonoid (mgQE/100g): 506.82, 309.69, 365.40 and 376.87, 241.20, 273.82
	Lemon peel in fresh (control), microwave and air oven drying in methanol and ethanol extract		Moisture (%):(81.23, 9.48, 9.58, protein (%): (11.53, 7.19, 7.06); fibre (%): 16.15, 12.35,12.47;ash (%):6.58, 5.92, 5.70; total phenol (mgGAE/100g): (1353.88, 1323.31, 1180.78) and (3251.53, 2632.81, 2504.4); DPPH (%): 79.37, 56.69, 50.93 and 65.56,56.01,52.64; flavonoids (mgQE/100g): 430.58, 442.79, 469.08 and 316.05, 317.41, 390.75
Ali *et al*., 2010	Rough lemon peel	Sudan	Ash (%): 2.04
Ali *et al*., 2016	Sour orange, sweet orange, grape fruit and lemon peel	Pakistan	Moisture (%):8,7, 6.8, 6.2 ; fat (%): 3.5, 3, 2.5 ,2 ; fibre(%):16, 18, 17.2 ,16.5; Ash (%): 8.3,7.2,6.9,6.3 ;protein (mg/100g):6.5, 5.2, 7.2,6.25; TSS (ºB): 0.80, 0.86,0.85 ,0.78; TA:1.06,1.10,1.2 and 1.11;reducing sugar(%):2.5, 2.0, 2.10 and 2.4 ;total sugar (%): (3.10, 3.0, 2.50 and 2.60); non reducing sugar (%): 0.60, 0.10, 0.45 and 0.20
	Grapefruit, Mousami Kinnow, Fewtrells Early, Malta and Eureka lemon (Ripe)		Moisture (%): 71.35, 72.42, 73.35, 70.65, 75.18, 75.39; Fat(%):1.68, 1.35, 1.59,1.35, 1.51 and 1.52; Fibre (%): 6.11, 5.53, 5.46, 5.30, 5.25, 5.15; Ash (%): 3.45, 3.51, 4.38, 3.79, 4.24, 4.57; Protein(mg/100g): 6.11, 5.53, 5.46, 5.3, 5.25, 5.15
Anhwange *et al*., 2009	Banana peel	Nigeria	Fibre(%): 31.70
Barros *et al.,* 2012	Lima orange, Peraorange, Tahiti lime, sweet lime, ponkan mandarin,	Brazil	Moisture (%):91.7,88.3, 88.5, 91.5, 77.1, 70.3, 66.6, 72.6, 79.3, 77.1; TSS (ºbrix): 7.7, 10.5, 9.8, 8.7, 11.0; Titrable

	lima orange, pera orange, Tahiti lime, sweet lime, ponkan mandarin fruit		acidity:0.23,1.25,4.40, 0.10, 0.84; TSS: acid : 34, 9, 2, 86,13
Bharali *et al*.,2017	Pomelo (white, pink, red) juice	Assam, India	TSS(°B):9.12,9.69,10.34; TA:1.03,1.08,0.87; Reducing sugar(%): 4.11,4.53,5.06; Non Reducing Sugar(%): (3.27,3.45,3.77);Total sugar (%) : 7.40, 7.98, 8.83 ; TSS/acid: (8.85,8.97,12.41) ViaminC (mg/100g) : 66.57, 53.68, 57.95
Bhatnagar *et al*., 2015	Nagpur mandarin juice	Rajasthan	Fruit weight (g):86.33 to 137.88g; TA (%): (0.72 to 1.53); TSS (°B): 7.66 to 11.83; Vitamin C (mg/100ml):34 to 41.83; Total sugar (%):5.75 to 8.87) Reducing sugar (%): 3.73 to 5.76
Boudries *et al*., 2012	Mandarin, Algerian Clementine cultivars (Rocamora, MermeCheryland, St. Martin, Cadoux, Monreal) pulp, juice	Algeria	Vitamin C(mg/100g):66.35,63.80, ,65.84,60.43, 47.97,55.12,65.33; TSS(ºBrix): 13.62,14.45,12.45,14.27,14.70, 11.83; TA:7.47, 6.67, 4.61, 5.93, 6.36, 7.40, 5.29
	St Martin, Cadoux cv, Cheryland cv , Monreal cv ; Mandarin cv, Rocamora and Merme cv pulp, juice		Phenol mg/100g: 3108.78,3144.24,3355.09, 3504.70,3888, 4006.45,4046.2 ;IC_{50} (mg/ml):1.91,1.83,1.80,1.77, 1.65,1.48, 1.14;Flavonoid (mg Catechin/100g): 946.42, 983.30,1030.77, 1068.53, 1078.92, 1088.33, 1281.98
Colecio-Juárez *et al*.,2012	Sweet lime at Stage I, II, III, IV in steam distillation and hexane extraction	Mexico	Titrable acidity:0.02, 0.01, 0.01, 0.01
Deshmukh *et al*.,2016	Khasi mandarin juice in immature and mature stages (500-600m,700-800m,900-1000m,1300-1400m) altitude	Meghalaya, India	Fruit weight (g): 134.2,136; 133.99,134.22;125.45,130.07;127.66, 132.11;TSS(°B):10.23,10.28;10.13,10.15,9.91,10.07,10.04 ,10.16; TA:0.77, 0.73; 0.81, 0.77; 0.88, 0.82; 0.92, 0.85); TSS/acidity: (13.22,14.15;12.60, 13.22 ;11.30,12.4, 10.98, 12.02) Vitamin C: 38.1, 36.11, 38.22, 38.01, 44.03, 42.64; 46.57,45.08; Reducing sugar: 3.89, 3.93; 3.75, 3.80; 3.82, 3.98; 3.89, 3.90; Total sugar (%): 5.94, 5.98, 5.92, 5.96; 6.07, 6.10; 6.01, 6.07
Dhuique-Mayer *et al*. 2005	Mandarin/Clementine group	Mediterranean	Vitamin C (mg/100g):40 and 52.8
Dipak and Ranajit,2004.	Pommelo juice and peel	Bangladesh	Vitamin C (mg/100g):26.36 and 19.34
Egbuonu and Osuji,2016	Sweet orange peel and seed	Nigeria	Fat (%): 6.27, 11.08; fibre (%):13.99, 2.98; ash (%): 4.89, 3.02; protein (%):11, 6.77 ; moisture(%):9.68, 6.32
Fatin and Azrina, 2017	Orange, grapefruit, lemon, lime, kaffir lime and musk lime.	Malaysia	Vitamin C(mg/100g):58.30, 49.15, 43.96, 37.24, 27.78 and 18.62; diameter (mm):65, 80,58,46, 50,30
Ghasemi *et al*., 2009	*Citrus sinensis* variety Washington,	Iran	Total phenol (mgGAE/g): (160.30,172.10, 170.50,195.50, 153.80,

	Citrus reticulata variety Ponkan, *Citrus unshiu* variety Mahalli, *Citrus unshiu* variety Sugiyama, *Citrus sinensis* variety Sungin, *Citrus unshiu* variety Ishikawa, *Citrus limon*, *Citrus reticulata* variety Clementine, *Citrus paradisi*, *Citrus aurantium, Citrus sinensis* variety Valencia, *Citrus aurantium* variety Khosheii, *Citrus reticulata* variety Page peel and tissue peel and tissue		148.8, 131.0, 161.7, 222.2, 223.2, 132.9, 164.7, 104.2)and (232.5, 197.4, 66.5, 140.9, 136.9, 144.9,102.2, 396.8, 112.1, 122.0,124.0, 90.3, 226.2); IC_{50} (mg/ml): (1.1,0.6,1.9,1.3, 1.7,1.8, 1.4,1.7, 2.1,1.9, 2.1, 1.8, 2.9) and (2.8, 2.2, 3.9, 3.6, 3.7, 3.8, 3.4, 3.2, 3.7, 3.9, 3.8, 3.5, 3.7); flavonoid(mgQE/g) :(23.2, 5.31.1, 19.8,2.1,4.8, 16.2, 5.7, 23.2, 7.7, 7.2, 7.9, 0.3) and (1.2, 0.6, 6.4, 2.2, 4.3, 5.3, 2, 17.1, 0.3,3.3,0.1,3.5,6.8)
Gorinstein *et al.*, 2001	Lemons, oranges and grapefruits juice, peel	Israel	Fibre (%): 14,13.9,13.9; total phenol (mg/100g): 190, 179 and 155; vitamin C (mg/100g): 59.8,59.6, 43.8
Gorinstein *et al.*,2004	Hybrid pulp and peel, white grape fruit pulp and peel	Israel	Total phenol ((µmol/g): 69.6, 171.9, 63, 134; flavonoid (mg catechin /100 g dw): 47.12 and 37.70
Hangsing *et al.*,2016	Khasi mandarin juice (Group I: 10 to 20 years II: 21-30 years; III:31-40 years; IV:>40 years age tree) (No stage mention)	Meghalaya, India	TSS(°B):10.98,10.36,10.62,10.52; TA(%): 0.81, 0.76, 0.78, 0.73; reducing sugar (%):7.84, 6.56, 6.51, 6.44);non reducing Sugar (%):1.05,2.76,2.49,1.15); Total sugar (%): (8.50,8.99, 8.68,7.27); vitamin C: (31.46, 28.49, 30.20,28.10) TSS: acid : (14.43,14.12, 13.30, 11.61); No of segment:(10, 10.40, 9, 9.20); fruit weight (g):(125.48, 130.69, 101.87, 80.59);diameter (mm): (65.04, 68.41, 59.06,55.25)
Wangchu *et al.*,2017	Pommelo juice	Arunachal pradesh, India	Fruit weight (g):343.07-2403.23;diameter (cm): 10.03 -18.17;No. of segments/fruit:12.67 to 18; TSS (°B):8.47-12.90; vitamin C (mg/100g):20.82 - 53.98; titrable acidity (%):0.37-1.27; reducing sugar (%):1.99 - 6.81; non reducing sugar (%):1.35 - 4.34; total sugar (%):4.60 - 9.45
Hashempour *et al.*,2013	Jaffa orange, thomson navel orange grapefruit, blood orange, satsuma sweet lime juice	Iran	Titrable acidity (%):1.53, 1.97, 2.26, 2.45, 1.57, 0.05; TSS (%):7.26,13.6,7.4, 8.10, 8.20, 7.06; TSS/TA: 4.76, 7.05, 3.27, 3.32, 5.42, 122.92; vitamin C (mg/100g):45.55, 62.77, 32.22, 42.77, 29.55, 38.33
Hiri *et al.*, 2015	Maltease orange peel	Tunisia	Moisture (%):76.01, protein(g/100g):8.12; ash: 3.17; fat:0.80; Vitamin C (g/100 g): 0.11
Hiri *et al.*,2017	Maltease orange peel by	Tunisia	Total phenol content (g GAE/100g): 1.97; total flavonoid content

	conventional solvent extraction		(g/100g):1.61
Ibrahim and Hamed, 2018	Orange and lemon peel in fresh and air oven drying	Egypt	Moisture (%): (74.35, 81.23; 9.96, 9.58); protein (%): (7.09, 11.53; 6.39,7.06); fibre (%) :(11.48,16.15; 10.46,12.47); ash (%): 4.21, 6.58; 3.51,5.71; total flavonoid content in methanol and ethanol extract (mg QE/100g): (506.82, 376.87; 365.40, 273.82) and (430.58, 316.05; 469.08, 390.75)
Igwe ,2014	Sweet orange, (*Citrus sinensis*), lemon (*Citrus limon*), grapefruit (*Citrus paradisi*) (unripe and ripe)	Nigeria	Vitamin C(mg/100g):50.10-59.00 and 43.00-49.50); (50.10 - 41.30 and 41.00 - 35.20), (44.80 - 36.40 and 34.20 - 24.00)
Jamil *et al*.,2015	Orange peel, lemon fresh sample	Pakistan	TSS (ºB):11.47,4.5, 4.5,16;
Janati *et al*.,2012	Lemon peel	Iran	Fat (%):4.98; fibre (%):15.18; ash(%):6.26; protein:9.42
	Orange peel		Ash (%): 5.51
Javed *et al*.,2014	*Citrus reticulata* var. Mandarin*;* *Citrus reticulata* var. Tangerine, *Citrus paradisi, Citrus sinensis* var. Malta*, Citrus sinensis* var. Mousami	Punjab	DPPH (%): 91.1, 88.0, 87.2, 86.0, 83.2
Kefi *et al*.,2016	Lemon peel (Eureka, Lisbon) juice and peel	Zambia	Vitamin C (mg/100g): (26.24, 33.28); moisture % :8.68,7.23; crude Fat (%):3.48, 2.46; crude fibre (%):12.40,14.20; crude protein (%):5.47,4.09; ash (%):4.53,5.08
Khatiwora *et al*.,2017	Lemon peel in methanol, ethanol, acetone, ethyl acetate extract in ripe and unripe fruit peel	Assam	Total phenol (mg catechol/g):395,340,295,190 and (367, 307, 210 ,155); flavonoid(mgQE/g):482,465,380,270 and 425,395,369,245
Kishore *et al.,*2010	Sikkim mandarin in north, east, south and west district, Sikkim juice	Sikkim, India	Vitamin C (mg/100g): 34.3, 39.6, 32.2, 28.5, 33.65 ; TSS(º Brix): 11.7, 11.40, 12.00, 10.70 ; titrable acidity: 2.20, 2.42, 1.74, 1.34; TSS: acid: 5.31, 4.71, 6.89, 7.98; reducing sugar (%): 6.0, 4.83, 6.2, 5.84, 5.70; total sugar (%):7.68,7.39, 9.49,7.55
Kishore *et al.,*2012	Dancy (*Citrus reticulata*), Thorny (*Citrus reticulata*), Fox (*Citrus reticulata*), Fortune (*Citrus reticulata*), Afourer (*Citrus reticulata*), Page (*Citrus reticulata*), King (*Citrus nobilis*) and Kinnow (*C. nobilis* x *C. deliciosa*)	Sikkim, India	Fruit weight(g): 98.5, 108.6, 90.3, 95.6, 87.6, 82.6, 64.5, 113.5; TSS(°B): 8.2, 8.8, 9.3, 8.4,10.4, 8.5, 10.2, 9.8; titrable acidity (%): 1.42, 1.12, 0.98, 0.96, 0.93, 1.21, 1.22, 1.28; TSS/acid: 5.77,7.85, 9.48,8.75,11.07, 7.02, 8.36, 7.65; vitamin C(mg/100g): 62.5, 55.2, 47.3, 48.2, 45.3, 48.4,52.7, 56.4; reducing sugar (%): 4.82, 5.1, 5.24, 4.65, 5.62, 4.75, 5.12, 4.95; total sugar (%): (6.32, 6.54, 6.84, 6.23, 7.86, 6.21, 7.42, 7.30)
Kumar *et al*.,2017	Pomelo (*Citrus maxima)* in Conventional Pasteurization and Microwave Heating Pasteurization	Dehradun, India	Moisture (%): (92.53,92.03) ; ash (%):(2.46,0.49) ; TSS (°Brix): (9.1, 8.8); titrable acidity (%): (1.40,1.68); reducing sugar (%): (3.59,3.50); vitamin C (mg/100g): (52.42,54.29); total phenolic

	(mature ripe)		contents (mg GAE/L): (690.5,705.3)
Kumar *et al*.,2013	Lemon, banana, jackfruit, sapota, pomegranate, carrot, beans, cabbage, cauliflower, potato (mature stage)	Karnataka, India	Vitamin C(mg/100g): 40.48,18.65, 20.76, 34.49, 34.14, 29.92, 23.58, 35.55, 39.42, 31.32
Kumar *et al*.,2013	Chakotra, lemon, mosambi, orange	Dehradun	Total phenol (mg/100ml): 16.08, 20.90, 23.07, 21.20 vitamin C (mg/100ml): 55.25, 67.97, 60.47, 60.20
Li *et al*., 2006	Lemon cv. Meyers, lemon cv. Yenbens, Grape fruit, Mandarin cv. Ellendale, sweet orange cv. Navelle	Auckland	Total phenol (mg GAE/g):60, 118,162, 121, 74
Mahajaan *et al*.,2018	Kinnow fruit juice (large, medium, small) size	Punjab	TSS(°B): (9.74,10.56,11.06); TA (0.68,0.84,0.96); TSS: acidity (16.00, 14.44, 13.36)
Mani *et al*.,2017	Pommelo (ACC-20)	Bengal	Fruit weight (g):1055.33
Mohammad *et al*., 2013	Rough lemon (peel, juice)	Sudan	Moisture (%): 18.35 peel, vitamin C (mg/100g): 70; ash:2.04 %
Mohamed *et al*., 2016	Grape fruit (white and red) peel	Sudan	Moisture (%):75.25,75.37; ash (%):1.5,1.6; protein (%):1.05, 1.15; fibre (%): 1.73,1.82; titrable acidity (%):0.16 and 0.22; vitamin C (%): 0.16, 0.15; reducing sugar (%):10.4,10.2; total sugar (%):19.78,18.89
Mohamed *et al.,*2018	Lemon (zest and flesh)	Tunisia	Total phenol (mg GAE/g): 204.40,105.55; flavonoid (mg QE/g): 27.50, 56.16
Mukhim *et al*., 2015	Assam lemon juice at 15,30,45,60, 75, 90, 110,120,130,140 150 days after fruit set (DAF)	Meghalaya	Vitamin C (mg/100g): 90.45, 56.45, 51.29, 51.39, 44.45, 36.11, 32.41, 30.56, 27.78, 11.11; TSS (ºBrix):6.73, 6.57, 6.53, 6.5, 6.47, 6.37, 6.30, 5.93, 5.93 ,5.57;titrable acidity: 1.17, 1.96, 3.65, 3.82, 3.86, 3.97, 4.18, 4.35, 4.46, 4.86; TSS: acid : 6.06, 3.59,1.79, 1.71, 1.68, 1.60, 1.51, 1.36,1.33,1.16
Nangbes *et al*., 2014	Sweet orange, lemon, lime juice	Nigeria	Vitamin C(mg/100g):50.23-56.26, 51.33-53.47, 34.12-35.62
Narjis *et al*.,2009	Orange peel	Baghdad	Protein (%): 4, moisture (%):11.86, Ash (%):5.34
Ndife *et al*., 2013	Orange juice (samples A, B, C and D)	Nigeria	Vitamin C(mg/100g):37.14, 42.57 23.01, 27.28
Nesrine *et al*., 2012	Mandarin (*Citrus reticulata* var. Tangerine) Thompson (*Citrus sinensis*) var Thompson Navel, Lemon (*Citrus limon*) var Lunari	Tunisia	Moisture (%): 79.16,75.82, 75.04; protein (g/100g): 1.78, 0.43, 1.46); fibre (g/100g):5.81,10.07,9.77); fat(g/100g): 0.62, 0.40, 0.49; ash(g/100g):0.82,0.83,1.17
Nweze *et al*.,2015	Apple, orange, pineapple and watermelon	Nigeria	Vitamin C (mg/100ml): 7.94,10.13,6.40,4.08
Oikeh *et al*., 2013	Sweet orange seed, flavedo and albedo of mature stage	Nigeria	Fibre (%):5.3%, 13.43 and 4.40; ash (%):2, 1.6 and 0.85; Protein(mg/100g): 6.13,3.94 and 0.88

Omoregha *et al*.,2018	*Citrus tangerine* and *Citrus sinensis* peel	Nigeria	Moisture (%): 65.20,69.30; Ash (%):0.48,0.49; protein (%): 1, 1.20; fibre (%):2.50, 2.63
Osarumwense *et al*., 2013	*Citrus sinensis* peel	Nigeria	Fat (%):10; Fibre (%):26.50; Ash (%):14.35; protein%: 4.05
	Citrus sinensis and *Vitis vinifera* peels		Protein(mg/100g):11.35, 3.73
Olabinjo *et al*., 2017	Orange (*Citrus sinensis*) rind	Nigeria	Fibre (%):12.79
Pallavi *et al*., 2017	Orange, Citron, Pomelo, sour orange, lemon peel and pulp extract	Karnataka	Vitamin C(mg/100g):52.67 and 25.9) ,(33.77 and 24.87),30.81 and 24.69 , (27.21 and 24.09),(20.21 and 23.93) ; total phenol (mg/g): (66.36 and 51.21; 48.5 and 28.34; 46.65 and 34.26; 41.5 and 37.72 ; 40.14 and 21.39;flavonoids(mg/g) :40.17 and 37.9; 21.61 and 20.75 ; 36.49 and 15.19; 39.23 and 29.83; 28.11 and 30.22
Paul and Shaha,2004	Orange, lemon, grape fruit, pommelo	Bangladesh	Vitamin C (mg/100g): 62, 37, 31,20; moisture (%): 87.1, 84.2, 90.7, 87; Fibre (%): 0.3,1.6, trace,1.6; protein (%):0.8, 0.9, 0.7,0.6; fat (%): 0.2, 0.8, 0.1,0.1
Peace and Happiness, 2017	*Shaddock (*Citrus maxima*)* peel and juice	Nigeria	Moisture (%):13.20 (peel) ,79.57 (juice); fat (%):9.74,0.83; fibre (%): 2.58, 0.32; ash (%): 2.49, 0.73; protein (mg/100g): 0.42, 1.76
Ramful *et al*.,2010	Lemons, mandarin, sweet orange peel	Mauritius	Total phenol (µg/g FW): 1882-2828 ,2649-6923, 4509-6470
Rangel *et al*.,2011	Acid lime i.e Tahiti lime in conventional and biodynamic juice	Brazil	Titrable acidity (%): 6.05, 5.98; TSS (°B): 8.42,7.63; Vit C (mg/ 100 mL) :22.86, 22.80
Rapisarda *et al*., 1999	Orange juice		Vitamin C(mg/100g): 41.7 and 78.14
Rima and Wafaa ,2014	Lemon varieities; Meyer, Interdonato, Monachello, Santa Tereza, Eureka juice	Syria	TSS (%): 6.1, 7.5, 7.3, 7.97, 7; TA (%): 4.77, 4.62, 4.81, 4.88; TSS/TA:1.28,1.62,1.52,1.44, 1.54; Vit C(mg/100ml): 25.47, 22.52, 24.79,31.11,27.49
	Sweet orange varieties*:* Washington, Cara Cara, Gillette, Newhall Valencia, Jaffa, Salustiana, Maourdi, Sanguinelli, Moroblood, Hamlin, Cadenera, Balady, Succari, Khettmali juice		TSS (%): 8.9, 8.9,8.7, 8.8, 10.5, 11.7, 9.5, 10.8, 10.6, 10.6, 11.3, 11.5, 11.5, 10.1, 9.9 ; titrable acidity (%): 1.8, 0.99, 0.84, 0.77, 1.26,0.85, 1.03,0.91 ,1.23, 0.53, 1.24, 1.65, 0.08, 1.64; TSS/TA: 8.9, 8.9, 10.36, 11.43, 8.33 ,13.76, 9.22, 11.87, 11.87, 20, 9.11, 7.82, 6.97,126.25, 6.04;vitamin C(mg/100ml): 41.15, 43.55, 39.41, 38.51, 45.73, 41,47, 39.88, 38.67, 36.59, 40.42, 42.70, 42.70, 42.68, 41.70, 37.58, 43.46
	Mandarin varieites: common mandarin, Mandalina, Clementine,		TSS (%):11.8,10.4,11.2,13.9,10.4,10.8,12.7,10.8,11.0,10.1,11.2, 9.5; TA(%): 1.01,1.18, 0.59,0.49, 0.82,1.43, 1.22,1.59, 1.19,1.62

	Nova, Carvalhal, Dancy, Klimntard, Fortune, Ortanique, Minneola, Ponkan, Satsuma juice		0.61,0.86,TSS/TA:11.68,8.81,18.98,28.37,12.68,7.55,10.41,6.79, 9.24,9.23,18.36,11.05; vitaminC(mg/100ml):18.66,19.55, 50.36, 17.82, 33.41, 35.2, 39.20, 39.11,24.00,31.50,18.60,28.44
	Grape fruit varieities (Marsh, Star Ruby, Red blush) Pommelo varieties (Pommelo, Red Pommelo) juice		TSS (%):10.2,11.3,9.9, 9.8, 9.9; TA (%):1.73 ,1.87,1.79,1.40,1.43; TSS/TA: 5.89, 6.04, 5.31,7,6.92; vitaminC (mg/100ml): 40.22, 41.33, 43.55,37.46,38.22
Rokaya *et al*.,2016	Mandarin juice at 1300 msl, 1000 msl, and 700 msl mature stage	Lamjung district	Fruit weight (g): (84.20,98.10,94.20); TSS (ºB): (11.63, 11.05, 10.82); titrable acidity (0.66,0.67,0.77); TSS/TA (17.76,16.52,14.34); vitamin C (mg/100g): (27.62,26.16,25.41)
Rowayshed *et al*., 2013	Pomegranate peel	Egypt	Fibre (%):11.2
Riaz *et al*., 2015	Orange juice (in early, late, mid stages)	Pakistan	Vitamin C (mg/100g):56.35-58.21, 46.50-48.00 and 49.55-52; TSS (ºBrix):8-8.5, 8.8-10.2, 9.9-10.5; titrable acidity : 0.9-1.4,0.6-0.8, 0.55-0.80; TSS: acid: 5.71-8.8,12.25-14.66, 13.12-18.8; total sugar (%):6.35-7,7.7-8.25, 8.8-9.6,
	Kinnow mandarin juice (in early, late, mid stages)	Pakistan	Vitamin C (mg/100g):49.6-52.1,42.15-45.3,41.5-43.1 TSS (ºBrix):8.8-9.8,11-11.8, 11.84-12; TA: 1.1-1.35, 0.7-0.8, 0.55-0.65; TSS: acid: 7.1-8.9,14-15.71,18.21-20; total sugar (%):7.5-8.2, 8.8-9.6, 9.2-10
	Fruetrell's early juice (in early, late, mid stages)	Pakistan	Vitamin C (mg/100g):43.92-48.3, 38.3-40.4, 38.1-40.25; TSS (ºBrix): 7.36-9.65, 8.6-9.65, 10.5-12.11; titrable acidity :0.65-0.8,0.6-0.65,0.5-0.6; TSS: acid: 9.2-14.84,14.33-16,19.1-21; total sugar (%):6.8-7.5,7.4-8.8,8.9-10
Sharma *et al*., 2015	Grape fruit cultivar: Star Ruby, Foster, Duncan, Imperial, Red blush, Marsh seedless, Variant 1, Variant 2, Variant 3, Variant 4 juice	New Delhi	Fruit weight (g) 530.77, 491,405, 410.03, 304.10, 398.87, 464.47, 354.63, 421.40, 358.83; diameter (mm):102.56,103.22, 93.63, 94.92, 83.11, 86.01, 100.33, 91.67, 97.81, 86.95; TSS(ºBrix): 6.90, 6.97, 7.30, 7.93, 8.50, 8.50, 7.63,6.90, 7.67, 7.40); acidity: 1.10, 1.08, 0.98, 1.10, 1.07, 0.94, 1.04, 1.08, 1.06,1.05
Shrestha *et al*.,2012	Acid lime high hill areas (>1200 m asl), mid hill areas (600 to 1200 m asl) and Terai (<600 m asl) peel, pulp, juice	Nepal	Vitamin C (mg/100g): 68.3, 62.2, 49.70; TSS (%):7.6, 7.7, 7.8; TA (%): 7.3,7.2,7.0
Shrestha *et al*.,2015	Lemon, bitter orange, sweet orange, pomelo, grapefruit and citron juice	Nepal	Vitamin C(mg/100g):40.35,30.60,60.25,27.45,42.23,20.32
Singh and Raj, 2018	Lemon peel	India	Vitamin C (mg/100g):129; crude fibre (%): 15.18
Tareen *et al.,* 2015	Orange, grape fruit, lemon, mango,	Pakistan	Vitamin C(mg/100g):12.78, 10.90, 12.68, 7.84, 9.31

	papaya		
Uraku, 2015	*Citrus sinensis* and *Vitis vinifera* peel	Nigeria	Moisture (%):9.78,6.52; Fat (%):10.34%,1.16%; Crude Fibre (%): 13.51,4.96; ash (%): 1.57,4.24; protein(mg/100g): 3.73,11.35
Varu *et al*., 2016.	Lemon peel	Gujarat, India	Peel moisture (%): 81.60
Verma *et al*.,2012	Nagpur mandarin	Rajasthan, India	TSS (ºB):10.04, titrable acidity (%):1; TSS/acid:10.19; vitamin C(mg/100g): 38.31; reducing sugar (%): 4.88; non reducing sugar (%):2.63; Total sugar (%): 7.50; fruit weight (g):115.24
Wanlapa *et al*., 2015	Durian and longing peel	Thailand	Peel starch (%):2.55, 0.59; Crude fat (%): 0.82 ,12.01
Yadav, 2014	Kinnow, Emperor, Clementine Monreal, Clementine Denule juice	Solan, India	Vitamin C (mg/100g):27.79,24.11,26.32,27.79); TSS (ºBrix): 11.61, 8.78, 6.63, 7.14; titrable acidity : 0.75, 0.74,0.77,0.70; reducing sugar (%): 3.36, 3.48,3.42,3.01; total sugar (%):5.45, 5.78,5.30,5.36; non reducing sugar (%): 2.09, 2.30,1.88, 2.36
	Sweet orange cultivars (Pineapple, Blood Red, Mosambi, Jaffa, Valenicia late, Valencia Campbell, Washington Navel, Sanguinella Moschata, VagnaliaApirino) juice	Solan, India	VitaminC(mg/100g):60.31, 55.83, 50.06, 48.28, 47.49, 42.72, 47.17, 46.01, 37.82; TSS (ºBrix): 8.87, 7.98, 7.52 8.28, 9.03, 8.58,8.90, 10.13, 6.97; titrable acidity: 0.55, 0.53, 0.58, 0.64, 0.58, 0.58, 0.56, 0.81, 0.59; reducing sugar (%): 3.36, 2.79, 2.77, 2.62, 2.55, 2.64, 2.88, 3.34, 2.95; total sugar (%):5.55, 5.79, 5.71, 5.49, 5.38 ,5.54, 5.62, 5.36, 6.18 ;non reducing sugar (%):2.25, 2.22, 2.26, 2.15, 2.09, 2.09, 1.95, 2.12, 2.98
	Kagzi lime and Baramasi lime juice	Solan, India	Vitamin C (mg/100g): 28.13 and 22.80; TSS (ºB): 13.86, 10.01; TA: 5.78, 4.63; reducing sugar (%):1.45,1.03; total sugar (%):2.55, 3.49; non reducing sugar (%):1.10,2.47
	Grapefruit cultivars 'Ruby Red', and 'Duncan'.	Solan, India	Vitamin C (mg/100g):52.42 mg/100 ml juice and 45.82 mg/100 ml juice; TSS (ºBrix):8.39,7.23; TA:1.12,1.14; reducing sugar (%):3.20, 1.94; total sugar (%):5.88,4.67; non reducing sugar (%):2.68,2.73
	Lemon cultivar kagzi kalan	Solan, India	Vitamin C (mg/100g):54.17 mg/100 ml juice; TSS (ºBrix):5.89; titrable acidity: 5.68; reducing sugar (%):2.64; total sugar (%):4.32; Non reducing sugar (%):1.68
Zaker,2017	Orange (Peel and pomace)	Parbhani, India	Moisture (%): 7.8,9.13 ; crude fat (%): 1.98, 1.53 ;crude protein (%):6.14, 7.34; crude fibre (%):11.14,6.20; ash (%): 3.81,3.36 ; total phenol(mg/g):106.5, 98.01; (acetone extract) ; 75.14, 39.12 (alcohol extract) ; vitamin C(mg/100g): 647,115.30

2.5. Essential oil and its characteristics

Essential oil is a group of volatile aromatic compounds produced by several plant species. It is the mixtures of many compounds consisting of isoprenoids, monoterpenes, and sesquiterpenes (Marin *et al*., 2007). Essential oil finds it application as pharmaceuticals, food flavour additives and natural antimicrobials amongst others (Bakkali *et al.,*2008). It can also be used in the manufacture of food and medicines and as a flavouring agents, cosmetics and domestic household products (Braddock *et al.,*1986). It exhibits antibacterial, antifungal, and insecticidal properties (Zoubiri and Baaliouamer, 2014). Extraction methods such as steam or hydro-distillation, water, steam and organic solvent extraction, cold pressing and supercritical CO_2 are applied. The range of oil content is between 0.5 and 5.0 % (w/v). It consists of high concentration of limonene, p cymene, and ocimene (20-70 %) as compared to other components which are present in trace amounts (Palazzolo *et al.,* 2013). Factors like nature of fruit, soil type, climate, genotype, age and extraction process affect the quality, quantity and composition of citrus peel essential oil (Dugo *et al.,* 2000; Palazzolo *et al.,* 2013).

Essential oil of citrus is found in the rind wall which can be extracted as a by-product during processing by hydro distillation in clevenger apparatus. The essential oil can be characterised by different physical and chemical characteristics.

The peel of citrus fruit is rich in essential oil which yields up to 0.5 to 3.0 kg oil/tonne of fruit. There is diverse use of essential oil such as in alcoholic beverages, confectioneries, cosmetics, pharmaceuticals and also for improving the shelf-life food stuffs safety (Wadhwa and Bakshi, 2013).

The weight, quality and purity of essential oil are determined by measuring the specific gravity which is reported to be in the range of 0.69–1.88 (Pedranti,2011). Most of the essential oils have the specific gravity less than 1 (Osagie *et al.,*1986), except few containing oxygenated aromatic compounds. Refractive Index (RI) determines the refraction of light which pass through the oil. RI gives purity test of oil and does not give percentage purity, Kumar (2014) have reported that the RI of 1.47 is categorized as highly pure oil in *Citrus sinensis* oil. Factors such as variety, presence of gums in peels, difference in ratio of albedo and flavedo layers, extraction methods, stage of maturity and harvesting determines oil yield and the yield and major constituents depend on the parts of the plants used for the extraction (Ahmad *et al*., 2016). The surface area of fruit and water supply during ripening could also increase the essential oil content (Bhuyan *et al*., 2015). Non rancid value determines the quality of oil Rethinam (2003), maximum value for which is 5.00 mg KOH/g fatty acid. Oil molecules are broken down into fatty acids, which make it more prone to oxidation and inturn less stable and lead to rancidity of oil. Oil bearing low fatty acid is considered to be of good quality.

Acid value of the oil is other parameters to be consider for quality if oil has low acid value it is applicable for making skin care products (Kumar, 2014). Further storage life of oil is more if oil has low acid value. Peroxide value gives extent to which oil has undergone primary oxidation. It shows rancidity of unsaturated fats and oils. Unsaturated oils are more prone to autoxidation. Peroxides are intermediates in the autooxidation reaction. The low peroxide value depicts more stability of oil and thus more storage. If the oil is stored properly its shelf life will be extended. As per the International Olive Council (IOC) standard is < 20 mEq O_2/kg oil. If peroxide value is higher than 20 mEq O_2/kg it leads to less stable oil with a shorter shelf life (Mailer and Beckingham, 2006).

The high saponification value of oil showed presence of high percentage of fatty acids in the oil (Omolara and Dosummu, 2009) and is due to presence of high proportion of shorter carbon chain lengths of fatty acids (Gohari *et al.,* 2011). If oil is having high saponification value, it can be used for soap making and for detecting oil adulteration. The relatively high value recorded means that it has potential for use in the industry (Akubugwo and Ugbogu, 2007). It is an indicator of the average molecular weight and chain length. It is inversely proportional to the molecular weight of the oil (Onwuka, 2005).

The iodine value determines the degree of unsaturation in oil as well as quantifying the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation. It can also be used in predicting the drying property of oils. Moderate iodine value oil can be used in industries as feed stocks. If there is low iodine value it means, oil has low susceptibility to oxidative rancidity (Fox and Stachowiak, 2007). Therefore, the lower the iodine value, the lower is the unsaturation and hence it reduces the tendency of the oil to undergo oxidative rancidity.

Table 2.2. Review on essential oil of different citrus germplasm

	Objective 2: Physico chemical characterization of essential oil														
Referrence	**Fruit**	**Place**	**Characterization of essential oil**												
			Yield (%)	**Color**	**Refractive index**	**Specific gravity (g/cm3)**	**(mg KOH/g oil)**	**Saponification value (mg KOH/g oil)**	**(mg KOH/g oil)**	**Free fatty acid (%)**	**Iodine value (g/100g)**	**Peroxide value (meq/kg)**	**Thiocyanogen value (%)**	**Phenol**	**DPPH (%)**
Adeniyl *et al*.,2018	Nigerian lime (*Citrus aurantifolia)* seed oil	Nigeria				0.86	1.86	185.30		1.37	107.8	15			
Ahmad *et al*., 2016	Malta (*Citrus sinensis*), Eureka lemon (*Citrus limon*), Mousami (*Citrus sinensis*), grapefruit (*Citrus paradise*), Kinnow (*Citrus reticulata*) and Fewtrell's (*Citrus reticulata*) peel	Pakistan	1.21,1.12 0.98,0.73 0.32,0.22												
Ali,2015	Orange, grape fruit and lemon peel	Pakistan	45,43,53			1.47, 1.48, 1.42		1.99, 1.79, 2.23	16.75, 13.5, 13.7			18.68 , 9.59, 13.67	18.68, 13.67 ,9.59		
Abubakar *et al*. 2014	Nigerian sweet orange seed	Nigeria					7.59	37.08				2.20			
Anwar *et al*.,	*Citrus limeta, Citrus*	Pakistan			1.46,		2.18,	180.90,			110,				

2008	*paradisi, Citrus sinensis, Citrus reticulata* seed				1.46, 1.46, 1.46		0.66 ,0.5, 1.30	198.85, 189.50, 186.0			101.5, 99.85, 104.80				
Baik *et al*., 2008	*Citrus Natsudaidai Hayata; Citrus tamurana Hort. ex Tanaka, Citrus tangerina Hort. Ex Tanaka, (Citrus unshiu×Citrus sinensis) ×Citrus reticulata Bujiwha; Citrus grandis Dangyuja; Citrus aurantium Jigak; Citrus aurantium; Citrus erythrosa; Citrus natsudaidai Hayata, Citrus benikoji Gamja; Citrus pseudo gulgul; Citrus paradisi Mac.×Citrus tangerina Hort. ex Tanaka Seminole; Citrus sinensis*, *Citrus platymamma*		0.6,2.1 1.4,1.4 0.8,0.9, 0.9,0.9, 1.2,3.5, 0.7,2.5, 1,1.4												
Bhuyan *et al*.,2015	Khasi mandarin (*Citrus reticulata*) Green mature, turning, ripe stage	Jorhat, Assam	6.9,8.1, 5.0		1.38, 1.47, 1.48	0.84, 0.84, 0.85	0.78, 0.69, 0.59		1.52, 2.95, 3.77						

Bourgou *et al*.,2011	Bitter orange, lemon, orange maltaise, mandarin Stage 1(green colour, immature), stage2 (yellow colour; semi-mature), stage 3(orange color, mature)	Tunisia	0.23,0.12, 0.46,1.30, 0.48,0.62 0.13,0.74, 0.52,0.22, 2.70,1.13												
Chaiyana *et al*., 2014	Pommelo sub sps KaoNamphung; KaoPuang; Kao-Tang-Gwa, and Kao-Yai	Thailand	0.18, 0.17, 0.18, 0.19												
Colecio-Juárez *et al*., 2012	Sweet lime (*Citrus limetta*) Stage I, II, III, IV in steam distillation and hexane extraction	Mexico			1.47, 1.47 1.47, 1.47 1.47, 1.47 1.47, 1.47										
Demery *et al*., 2015.	Cumin seed oil and orange peel oil	Egypt		0.02	1.47, 1.47		0.56 ,0.28	128.80 ,98.62	128.24 ,98.34	0.28 ,0.14	156.1 168.2				
Esfahani and Moradi, 2017	Lime fruit (*Citrus aurantifolia*) immature, semi mature and mature stages	Iran	1.54, 0.80, 1.23												
Enis *et al*., 2019	*Citrus aurantium* Leaf, flower and peel	Tunisia	0.12, 0.40 and 1.35												
Evbuomwan	Grapefruit,	Nigeria	9ml,7ml		1.48,	0.85,	15.9	191.4l,							

et al., 2016	Tangerine peel				1.47	0.84	9,1.96	182.7							
Njoku and Evbuomwan , 2014	Nigerian orange, lemon and lime peel	Nigeria			1.47, 1.48, 1.48	0.84, 0.85, 0.86	1.99, 1.77, 2.20	16.19,13.76,13.23				68.75, 13.57, 9.57			
Fakayode and Abodi, 2018	Orange (*Citrus sinensis*) peel Soxhlet method	Nigeria			1.47	0.84	3.71	188.00		1.86	82	16			
Guneser *et al*., 2018	Lemon seed, Orange and grape fruit seed oil (Cold pressed)	Turkey			1.47, 1.47, 1.47	0.94, 0.92, 0.92		199.95, 203.89, 202.55		0.60, 0.27, 0.37	117.5 117.9, 125.01	9.40, 13.10, 13.80			
Hashmi *et al*., 2012	Sweet orange	Maharashtra				0.84						3.50			
Jalgaonkar *et al*.,2012	Jatti Khatti, Soh sarkar, grape fruit, pummelo	IARI, New Delhi	8.08, 4.64, 4.78, 3.79												
Javed *et al*., 2014	Mandarin Mousami Grapefruit, Malta Tangerine	Pakistan	0.45,0.37, 0.33,0.30, 0.28		1.46 1.47 1.48 1.47 1.47	0.84, 0.85 0.86 0.85 0.84									
Kamal *et al*.,2011	*Citrus reticulata*, *Citrussinensis* and *Citrus paradisii* Fresh, air dried, oven dried	Pakistan	(0.30,0.48,0.50) ; (0.24,0.5,1.07) ; (0.20,0.3,0.40)		(1.46, 1.46 1.46); (1.46, 1.46, 1.46); (1.46, 1.46, 1.46)										

Kashaf *et al*., 2013	*Citrus paradisi* (Grape fruit) peel oil solvent extraction method	Pakistan			1.47	0.85	0.81				99.40				
Khan *et al*., 2012	Jaffa, blood red, mosambi, kinnow, grapefruit	Pakistan	0.25,0.21 ,0.19, 0.04,0.13												
Khan *et al*., 2013	Sour orange and sweet orange	Pakistan						171,183		5.8, 2.3	100, 103	35.2,1 3.5		6.18, 7.78, 7.21, 5.20, 8.58	2.18, 1.84, 1.01, 1.01, 1.79
Mohammad *et al*.,2014	Olive oil, sunflower oil, melon seed oil	Bangladesh											75-83, 78.4-81.3, 68		
Moosavy *et al*., 2017.	*Citrus limon*	Iran	1.22											81.82	55.09
Musa,2017	*Citrus paradisi* seed oil, *Citrus sinensis* seed oil	Sudan				0.91, 0.91	9.46, 9.36	270, 312.7	56.85, 51.54		66.49, 60.90	20 ,19			
Nwobi *et al*., 2006	African sweet orange seed oil	Africa			1.47	0.92	82%	192			108	92.84			
Ohloff, 1990	Orange, bergamot and petitgrain, mandarin oil orange flower Steam distillation Cold pressed	Pakistan	0.5,0.5 ,0.5,0.2												
Okunowo *et al* 2013	Grape fruit (*Citrus paradisi*) peel	Nigeria	0.79			0.88									
Olabanji *et al*., 2016	*Citrus sinensis* seed and peel oil	Nigeria			1.46, 1.47	0.99, 0.78	23.6, 25.1	222.58, 41.25	178.24, 28.96	11.86, 12.61	78.83, 120.1	18, 5.40			

Ramadan *et al*., 2018	*Citrus sinensis* peel oil Soxhlet apparatus	Syria			1.49, 1.49		23.4, 26.5	141.35, 134.63	117.96, 108.19	11.74, 13.27	75.68, 77.85				
Saidat *et al*., 2018	Orange peel (steam distillation, distillation and solvent extraction	Nigeria	4.40,3.47 , 2.54			0.84	3.88	43.71	39.83	1.94	98.16	12.06			
Sharma,2016	Kinnow peel physical method and hydro distillation, physical method, fresh peel by hydro distillation, Mechanical dried peel powder by hydro distillation, Solar dried peel powder by hydro distillation	Solan, India	0.29,0.18 0.37,0.91 ,0.62		1.48, 1.36, 1.44, 1.35, 1.38	0.81 0.80 0.86 0.83 0.85	3.46, 5.02, 4.07, 4.34, 4.46	186.43, 181.73, 186.90, 183.88, 184.55	182.97, 176.71, 184.89, 179.55 180.09	1.74, 2.53, 2.05, 2.18, 2.24					
Singh and Raj,2018	Lemon peel (distillation and solvent extraction)	Lucknow , India			1.47, 1.47	0.85, 0.84	4.52, 5.21	146.65, 149.65		1.26, 1.46	73.29, 76.29	5.37,5 .67			
Subramanian *et al*., 2014.	Grape fruit peel	Coimbatore						194.40							
Tobias *et al*., 2011	Nigerian sweet orange (*Citrus sinensis*) peel	Nigeria			1.47	0.84	12.34	177.03							
Weiss, 1997	Sweet orange, eureka lemon, mandarin and bergamot orange	Australia	0.80,0.90 ,0.80 0.55												
Yadav *et al*., 2017	*Citrus paradisi* peel	Allahabad India													56.07

Zaker,2017	Orange (*Citrus sinensis*) peel	Parbhani India	0.55 ml		1.49	0.85	0.28	7.61			120	<0.5			
Zohra *et al*., 2015	Algerian species of Citrus i.e. orange, lemon, mandarin and Bigaradier leaves	Algeria	0.96, 1.02, 0.51, 0.73												

2.6. GCMS analysis

Gas Chromatography Mass Spectrometry (GC-MS) is a technique which is used to determine the chemical composition of the oil using the test sample. It has become an important approach for monitoring the organic pollutant in the environment (Bliesner, 2006; Amirav *et al*., 2008)

This is a common type of chromatography being used in analytical chemistry for separating and analyzing compounds which are present in the given sample (Burfield, 2005).

The development of chromatographic techniques has allowed us to make considerable progress in the study of the chemical composition of essential oil. Gas Chromatography (GC) is, by all means, the best method, due to its simplicity, rapidity and efficiency, for both the identification and quantification of essential oil components and composition variations.

GC coupled with mass spectrometry proved to be a useful application to identify variations in the composition of essential oil and make comparative analysis (Chamorro *et al.,* 2012)

Table 2.3. Review on GCMS analysis in different citrus germplasm

Authors	Fruits	Compounds (%)																							
		thujeneα –	pineneα-	Sabinene	β-pinene	ocimeneB-	Myrcene	Octanal	Limonene	γ-terpinene	α -terpinene	Linalool	Terpine n-4-ol	Geranial acetate	Neryl acetate	β- caryophyllene	Citronellal	Neral	Geraniol	Geranial	Decanal	Nonanal	α-terpineol	trans-α-bergamotene	No)Other detected
Ali, 2015	Orange, lemon & grape fruit		4.45 3.20 10.40		- 3.82 23.12				54.15 64.00 17.07							- - 4.80								- - 4.67	**9,9,19**
Ascrizzi *et al.*, 2018	*Citrus limon* peel	0.4	1.6	2	10.6	0.2	1.6	0.2	50.2	9.6	0.3	1	0.6	0.4	0.6	0.3	0.4	5.7	0.8	7.1	-	0.5	0.6	0.4	**10**
	Citrus sinensis peel	-	0.6	2	-	3	2.2	2	85.7			3.5	0.3				0.1	0.4		0.6	0.6	-	0.5	-	**5**
Bourgou *et al.*, 2012	*Citrus aurantium* cv. Larange; *Citrus limon* cv. Beldi; *Citrus sinensis* cv. Lsenas four; *Citrus reticulata* c.v Elarbi (green colour, immature, yellow colour; semi-mature and orange colour peel	(0.14, 0.38, 0.37, 1.31), (0.27, tr,tr, 0.12), (0.44, 0.34, 0.20, 0.39)	(0.13,1.29, 0.41, 0.03), (0.03,5.90, 0.44, 0.03) (0.36, 1.14, 0.70,0.03)	Tr,6.48, tr,1.31), (Tr,3.8, tr,0), (0.20, 5.82, 0.36, 0.18)	(0.11, 1.06, 1.54, 0.43), (0.07, 31.49, 1.80,0.06), (0.38, 0.63, 0.97, 0.75)		(0.09, 1.54, tr,1.59), (0.48, tr, tr, tr), (0.07, 0.99,0.71,0.98)		80.5, 68.08, 86.43, 65.37), (67.90, 37.63, 81.52, 51.81), (90.95, 69.71, 85.35, 69.0)		(1.23,0.24, tr, 0.30), (0.91, tr, tr,1.52), (1.66, 1.05, 0.93, 0.73)														
Bousbia *et*	Lime *(Citrus aurantifolia),*		1.94, 1.75,		13.09, 15.35,		1.46, 1.33,		63.44, 65.25,	11.17, 8.08,		0.36, 0.18,													**21,23,19, 19,13, 17,19**

al., 2009	Eureka *(Citrus limon),* Villa Franca *(Citrus limon),*Marsh seedless *(Citrus paradisi),* Tarocco *(Citrus sinensis),* Valencia late*(Citrus sinensis),* Washington Navel *(Citrus sinensis)* ,Tengelo Seminole *(Citrus paradisi)*		1.18, 0.45, 0.49, 0.51, 0.53, 0.83		7.08, -, -, -, -,0.38		1.52, 1.81, 1.87, 1.85, 1.76, 1.79		71.22, 94.21, 94.68, 95.48 93.16, 90.34	6.58, 0.03, 0.05, 0.06, -,3.78		0.38, 0.18, 0.17, 0.30, 0.27, 0.62													
Chun Yan *et al*., 2010	Lemon peel oil		0.07		0.51				4.25							0.15									**51**
Colecio-Juarez *et al*., 2012	*(Citrus limetta)* Intense green,Green, GreenYellow and intense yellow peel	-	0.77,0.89, 0.82, 0.89	1.85, 2.12, 1.86, 0.93	8.63, 8.96, 7.62, 6.48		1.15, 1.31, 1.43, 1.68		66.8, 71.7, 74.4, 77.7			5.92, 3.61, 2.89, 1.98	- ,0.07, 0.05, 0.06	- ,0.26, 0.10, 0.20				0.2, 0.2, -, -		-, 0.18, 0.18, 0.22		-, -,0.03 ,0.04	0.33, -, -, -		**23,25,24, 23**
Darjazi , 2014	*Citrus limetta* peel; *Citrus aurantifolia* peel from two location	0.35; 0.35, 0.33	2.03; 1.66 , 2.32	2.00; 2.72 ,0.23	11.76; 10.19 , 16	0.25; 0.24 , 0.37	1.45; 1.45 1.34	0.02;0.07,0.15	60.81; 64 , 52	8.94; 8.83, 9.30	0.28; 0.48, 0.23	0.37; 0.31 , 0.52	0.23; 0.44 , 0.51	0.31	0.35	0.21; 0.3 , 0.46	0.04; 0.15, 0.18	0.58;0.91,1.21	0.11; 0.12 , 0.18	10.98; 1.50, 1.74	0.05; 0.11, 0.21	0.04	0.50; 0.72, 0.88		**10, 28,17**
Das *et al*.,2014	*Citrus reticulata* peel	-	2.32	-	-		4.17		45.68							0.02					0.54				**43**
Demery *et al*.,	Cumin seed	0.36	0.83		14.30		1.07		-	19.28	0.22		0.25												**34**

2015	0range peel		0.55	0.54			1.96		95.03	0.10		0.66													**7**
Deng *et al.*, 2017	*Citrus medica* peel	0.55	3.72	0.06	1.95	0.6	1.78	-	60.13	6.17	-	-	0.07	0.1	0.06	0.01	-	-	-	-	-	-	0.06	0.39	10
Dharma wan and Kasapis, 2008	*Citrus nobilis* variety *microcarpa*, *Citrus sinensis* and *Citru s reticulata* peel	tr,tr, 0.2	0.6,0.5 , 0.9	0.1 0.3 0.1	0.2, tr,0.4		2,2,1.9		96.3, 95.6 ,9 0.8	-, 0.4, 4.3		tr, 0.2, 0.3			Tr,-tr		Tr,tr,tr	Tr,tr, tr		Tr, tr, tr	0.1 , 0.1 , 0.1	0.2, 0.1, 0.3	Tr, tr,tr		**17,8, 21**
Enis *et al.*,2019	*Citrus aurantium* (leaves, *flower* , *peel*	10.65 , 6.15, ≤0.1	0.68, 0.77, ≤0.1	-	-	≤ 0.1,0.11, 1.12	≤ 0.1, ≤ 0.1, 0.9	-	6.52, 5.03, 73.6	Less than 0.1		37.24, 41.82, 48				1.00, 0.45, 0.55	0.21, ≤ 0.1, ≤0.1	3.40, 4.8 ,3.26	-	-	-	-	-	-	**53**
Esfahni and Moradi, 2017	*Citrus aurantifolia* immature, semi mature and mature stage peel	0.16, 0.20, 0.33	1.15, 1.31, 1.67	- ,2.48, -	17.81, 16.75, 24.25	0.53, 0.45, 0.37	1.06, 1.02, 0.94		39.38 , 36.23 , 32.81	3.81 4.96 8.92	0.27, 0.32, 0.50	1.39, 1.23, 1.31	-	0.35, 0.35, 0.24	0.09, - ,0.12	0.29, 0.61, 0.24	0.06, -, -	11.01, 8.82 ,6.9 9	1.04, 2.94, 1.04	14.32, 11.53, 9.31	-	0.10, -, 0.19	2.06 ,2. 17, 3.02	0.13, 0.28, 0.16	**10**
Goyal and Kaushal, 2018	*Citrus reticulata* peel	-	0.27	0.08	-	0.12	3.03	-	50.42	0.05		0.45	0.45		0.06	0.34	-	-	-	-	1.28	0.17	1.19	0.06	**66**
Hamada *et al.*, 2017	*Citrus sp. Shiiku, Citrus keraji, Keraji, Citrus rokugatsu, Citrus nobilis, Kunenbo, Citrus keraji Kuriha peel*	T, 0.38 0.71 0.46 2.01	0.62 1.46 2.50 1.78 5.09	0.20 0.24 0.27 0.26 0.55	3.04 0.86 1.66 1.09 3.56	0.94 - t - -	3.45, 22.68 3.51, 25.41, 3.50	- 0.18 - - 0.49	32.51 35.67 46.70 44.65 40.87	t, 6.37 11.5, 67.28 24.22	-f t 0.26 0.95	t 21.94 3.34 7.27 0.31 1.66	0.64 t 0.60 0.21 0.70			- 0.51 - - t	- 0.90 - - -	t 0.73 t - -			- 0.46 - - 0.62	- t - - t			**35**
Hamdan *et al.*, 2013	*Citrus reshni* peel and leaf	0.09, 0.87	1.11,2. 03	0.98, 22.27	-,0.03	0.45, 5.39	3.45, 2.91		79.46,3 .78	-,3.4 3	0.01, 1.41	3.26, 23.91	0.52,6.32				0.02, tr		0.07, tr	0.29, 0.04	1.01, -		0.84, 1.34		**52, 97**
Hatil *et al.*, 2015	*Citrus paradisi peel*		3.74	1.21			12.85		74.45												1.18				**6**

Jalgaonkar *et al.*,2013	*Citrus jambhiri*, *Citrus karna* ,*Citrus paradise*, *Citrus grandis*	-	0.48 0.47 0.11 0.25		0.74 0.10 0.07, -		2.17, 2.10, 1.62, 1.80		95.13 96.23 93.57 96.41	0.17 0.19 0.15-		0.11 0.18 0.30 0.23	0.10 0.13 0.05-	0.05 0.12 0.13, -	-	0.04 - 0.09-	-, -, 0.26 0.17	-,-, 0.18 0.06	0.04, 0.15 0.07-	0.08- 0.19-			–0.18-		**3,1, 3,0**
Javed *et al.*, 2014	Mousami, malta, grape fruit, mandarin, tangerine								87.84, , 89.84, 87.45, 58.50														12.16,8.45,10.16,12.55		**2,3,0,0, 4**
Kamaliroosta *et al.*, 2016	Orange, tangerine, sweet lemon peel, sour lemon						-. -,0.88,1.95		64.87, 28.10,41.79,83.03			-, -,1.80,													**6,15,17,6**
Kamal *et al.*, 2011 (Fresh, ambient dried and oven-dried peels)	*Citrus reticulata*,	-	1.27, 1.62, 1.67	-,0.49, -	0.40, -, -	-, 0.28, -	3.27,4.05, 4.02	-,1.06, 1.15	69.9, 64.1, 71.1	- 0.23, -		1.10, 2.00, 2.56					0.78, 0.45, 0.58				2.33, 7.71, 5.80		-, 1.10, -		**15, 23,9**
	Citrus *sinensis*		-	1.65, 1.77, 2.48	0.37, -,0.62		0.34, -, -	4.19, 3.76, 4.41	80.9, 72.7, 66.8				1.52, 2.10, 1.60					0.71, 1.24, 1.60				1.02, 0.71, -		-, 2.34, 3.10	**21,22,22**
	Citrus paradisii		-	1.19, 1.26, 1.50	0.52, -, -			3.51, 3.57, 3.60	50.8, 53.8, 65.5		0.91, -,	-	1.07, 1.55, 1.04	-				1.78, 1.48, -				2.29, 2.50		-, 1.33, 2.68	**35,36,37**
Khan *et al.*, 2012	*Citrus sinensis* variety Jaffa, *Citrus sinen sis* variety Blood Red, *Citrus sinen sis* variety Mosambi), *Citrus reticulata* variety Kinnow, *Citrus para disi* variety Shamber peel	-	nd,nd, nd, 1.6, 1.3				7.2, 3.7, nd, nd, 3.6;3		76.0, 74.4, 69.1 75.7, 40.9			2.0 ,3.5, 3.5, nd, 1.0							nd, nd, nd, nd, 6.7						**12, 10, 12, 9,14**
Mahmud	*Citrus acida*										0.61														**17**

et al., 2009	peel																								
Majnooni *etal*., 2012	*Citrus aurantium* leaves	-	0.5	0.1		0.25	2.37		57.57	0.11		8.01	0.16				0.89	0.22	0.57				0.65		**27**
Njoku and Evbuomwan,2014	Orange, lemon and lime peel	-	4.45 3.20 10.40	-	- ,3.82, 23.12		- ,- ,0.73		54.15 ,64.00 ,17.07							-, - ,4.80								-, - ,4.67	**9,9,18**
Nongalleima *et al*., 2017	*Citrus macroptera* peel	tr				0.17		0.82													tr	tr	tr		**51**
Oladipupo *et al*.,2014	*Citrus aurantifolia* and *Citrus reticulata* leaf from two localities	-,0.3 , 0.6 -	7.9 - 3.1 -	- 0.2 - -	1.1 ,0.2 0.8 -	2.1 - 4.5 -	0.1 ,1.6 -	-	- 44.7 - 12.2	0.4 - 1.2 0.9		0.2, 1.3 4.2, 14.5	- - - 1.5		- 4.7 - -		- 4.0 - 38.1		1.2 - -	- 38.2 - -				1.0 - - -	**7,3,15,4**
Owolabi *et al*., 2018	*Citrus limon* peel and leaf	-	1.1, 1.2	3.9,15.9	-	0.4,3.9	3.1,2.9	0.1, -	85.9, 31.5	0.2,0 .9		0.5, 4.6	0.9, 1.4	0.1, 3.4			0.5,11. 6	0.3, 4.5	0.2, 1.3	0.4, 4.5	-,0.2	0.1, -	0.6, 0.6	-	24
Prasad *et al.,* 2016	*Citrus maxima* Leaf & rind				-,2.25	-,0.33	-,2.06		-,89.04			-,0.49	0.19,0.41			16.89.-		-,0.18		-,0.13		1.45	-,0.39	0.74, -	**23,6**
Rana and Blazquez, 2012	*Citrus macroptera, Citrus maxima* pink and white peel	-	0.4, 0.2, 0.3	0.2, nd, 0.1	0.1, nd, 0.7		0.8, 0.8 ,0.1		55.3 ,72.0, 80.0	0.6, 0.3 , 0.3		1.1, 1.5, 0.7	0.4,0.3,0.3			4.7, nd ,nd		2.2, nd, nd	1.3, nd, nd	3.5, 0.6, 0.4	0.3, nd, nd	0.4, nd, nd	2.3,1.3, 0.7		**26, 13, 9**
Sikdar *et al.,* 2016	*Citrus sinensis* peel		1.24				3.79		94.13																**1**
Varkey *et al*.,2013	*Citrus aurantifolia* peel from five location	0.38, t, t, 0.38 1.24	1.85, 1.39, 2.02 1.86, 2.46	1.73 ,2.45, 2.70 1.74, t	12.78 , 14.30 15.39 , 12.87 13.99				40.00, 41.62 37.8, 40.25 41.93		12.29 ,10 .59 8.02 ,12. 36 10.63		1.17, 1.23, t 1.18 ,1.03										t , 2.02, 2.26, t,t		**27,19, 22, 25, 23**
Wang *et al*., 2013	*Citrus reticulata variety* Chenpi and	0.12 , 0.03 0.12; 0.22,	1.22 ,0 .48 1.05 ;1 .29	0.01, tr ,0.04 ;0 .15, 0.12 ,0.2	0.53, 0.15 0.47; 0.58		1.33, 1.7 ,2.77 ; 2.86, 2.49 ,2.53	0.05, 0.15 0.08; 0.13	58.91, 55.11 74.08 ; 77.66	6.72, 6.58 7.84; 6.99	0.05, 0.33 0.21 ;0.19 0.18 ,0.2	1.29, 1.19 3.39; 3.12,3.9,													**56, 66,55,40, 35, 48**

	Qingpi peel *each in three places*	0.15, 0.24	0.89 ,1 .26		0.48, 0.65			0.30 ,0. 64	77.63, 70.55	7.28, 7.09	1	7.77													

Tr= <0.1 % , - = not detected , Nd= not detected

2.7. Pectin

It is a natural product which can be found in the cell walls of all higher plants and is being used for gel formation, thickening and stabilizing properties in a wide range of application in food as well as pharmaceutical and in cosmetic industries. It can be used for gel formation, thickening and stabilizing properties. Citrus waste is rich in oil, pectin and several by- products. The failure to reuse such material ultimately results in the waste and depletion of natural resources. Fruits, apple and citrus wastes are the main source of commercial pectin (Thibault and Ralet, 2003) usually generated after juice extraction in the industries. Measures of extraction like oxalic, hydrochloric, nitric and sulphuric acid) at high temperatures (80-90°C) are applied for pectin. The range of pectin is from 20 to 30 % of dry matter waste in citrus fruit. It can be used in juice-based drinks and as stabilizer in acidic protein beverages. Pectin can be used in confectionery jellies to give a good gel structure. In 0.5 up to 1 % of pectin is desired in food preparation. It can also be used in pharmaceutical and cosmetic products (Van Buren, 2012).

The functional behaviour of pectin depends upon the equivalent weight. High equivalent weight showed higher gel forming effect while low equivalent weight means higher partial degradation of the pectin which is disadvantageous (Yadav *et al.,* 2017).

Methoxyl content is an important aspect for determination of the gel formation capacity which decides the setting time and gelling ability of pectin (Constenla and Lozano, 2003). Spreading quality and sugar binding capacity of pectin is increased with increase methoxyl content (Madhav and Pushpalatha, 2002). Low MeO pectin will form a thermo-irreversible gel which means it will remain gelled even when heated at temperature that would melt it (Yapo and Koffi,2014). They are used in the food industry to make low-sugar jams because it does not require high-sugar levels to gel and is being utilized for pastries and molecular recipes designed not to be very sweet. They are used as a gelling agent, thickening agent, and stabilizer and can also be used as a replacement of fat in baking products and also to stabilize acidic protein drinks such as drinking yogurt (Tiwari *et al.,*2017).

The anhydrounic acid (AUA) denotes for the purity of extracted pectin and its value should be less than 65 % (Food Chemical Codex, 1996). Low value of AUA is directly linked with pectin which is having a high amount of protein, starch and sugars in the liberated pectins (Ismail *et al*., 2012). According to Food Chemical Codex (FCC), Food and Agriculture Organization (FAO), and European Union (EU), pectin must consist at least 65 % of galacturonic acid (Willats *et al*., 2006). Lower degree of esterification (DE) of pectin makes the fruit softer during maturation depending upon the species, tissue and stages of maturity (Sundar Raj *et al*., 2012). If pectin is high methoxyl pectins (HMP) (with DE > 50 %) it will require a high concentration of soluble solids while low methoxylpectins (LMP) (with DE < 50 %) form rigid gels by the action ofcalcium or multivalent cations, which cross-link the galacturonic acid chains (Garna *et al*., 2004).

Increased DE values will increase the intensities of band area of esterified carbonyl groups (Gnanasambandan and Proctor, 2000) during FTIR analysis. Further, DE in pectin can be determined by peak area in relation to the free carboxyl groups (1650 cm^{-1}) and esterified groups (1750 cm^{-1}). However, two peaks showed difference in their intensity ratio based on their extraction method.

Table 2.4. Review on pectin analysis in different citrus germplasm

References	Fruit	Place	Parameters reported													
			Qualitative tests								Quantitative tests					
			Color	Solubility				Sugar & organic acid	Yield in dry basis (%)	Yield in wet basis (%)	Eqwt (mg/ mol)	MeO (%)	Moisture content (%)	Ash content (%)	AUA (%)	DE (%)
				Cold water	Hot water	Cold alkali	Hot alkali									
Ahmed and Sikder , 2019	*Citrus limon var.* Ginger lemon, Cardamom lemonand China lemon	Bangladesh							5.71,8.08 , 12.73		298, 532, 301	3.97, 9.35, 2.86	12.94, 14.16, 10.43	4.15, 2.61, 4.02	82.2 85.45 74	27.38 ,62.12, 21.96
Aina *et al.*, 2012.	*Citrus limon*, (*Citrus paradisi* & *Citrus sinensis*	Nigeria	Brown brown brown	Insoluble, insoluble, dissolves slightly	Dissolve sdissolve s dissolves	Yellow precipitates yellow precipitates yellow precipitates	Dissolves and turned milky, dissolves and turned milky, dissolves and turned milky		2.76, 1.68, 2.31	16.71, 15.92, 15.70	694.44 534 293.60	4.46, 5.79, 3.90	66.6, 95.25, 80.95	30, 35, 34.50	-	-
Azad *et al.*,2014	*Citrus limon* pomace in premature, mature and over ripen stage	Bangladesh							13.13, 10.83 and 10.33		1175 1632 368	10.25 4.24 4.26	10.92 11.49 13.40	2.41, 2.46, 4.06	73.22 34.12 71.99	79.51 70.39 33.59
Bagde *et al.*,2017	Orange & lemon peel	Yavatmal	White, yellow	Insoluble, Dissolved slightly and form suspension	Dissolve sdissolve s	Yellow precipitates yellow precipitates	Dissolves & turn milky, Dissolves & turn milky		0.8, 0.2	8,6	100, 86.87	4.23, 5.32	66.60, 95.25	30, 35	-	-

Balachand *et al*.,2015	Sweet orange peel	Maharashtra	Brown	Insoluble	Soluble	Soluble	Soluble						82	0.76		
Devanooru *et al.,* 2015.	Orange peel	Karnataka	Orange	Insoluble	Sparingly dissolves	Sparingly dissolves	Partially dissolves	30			200	33.11	28	2		
Devi *et al*.,2014	Lemon peel with citric & nitric acid	Allahabad India									312.5, 833.33	6.2, 5.27	5.2,7.6	7.5, 3.5	91.52, 51.04	38.46, 58.62
Girima and Woku, 2016	Banana, Mango peel	Ethiopia									925.01 895.00	6.09 8.89	8.94 8.82	0.47, 0.92	53.6, 70.65	64.50, 72.17
Kanmani *et al*.,2014	*Citrus sinensis, Citrus limetta Citrus limon*	Coimbatore, India	Brown yellow yellow	Insoluble insoluble, soluble	Dissolves Dissolves dissolves	Yellow precipitates yellow precipitates Yellow precipitates	Dissolve & turn milky, Dissolve, Dissolve				594.86, 386.45, 253.70	6.84, 4.46, 2.35	58.72, 75.80 82.70		68.74, 42.80 39.48	3.50, 2.98 1.50
Rose &Abilasha, 2016	Lemon peel(pH 2) Temp60,70, 80ºC	Tamil Nadu								26.2, 28.0 and 29.8%	510	5.40		6.66	65.40	20.07
Roy *et al*., 2016	Pomelo peel pH 1 and 2									16.07, 16.74	540.04, 711.33			5.50, 5.69	84.29 , 85.57	61.19, 70.79
Teresa *et al*.,2017	*Citrusx lantifolia* Acid hydrolysis +alcohol precipitation , Acid	Mexico								36.45 34.86 36.21	400.37 , 622.25 706.74	10.12, 11.29 9.00 ,	7.9, 8.17 10.72			52.05, 64.09, 62.41

	hydrolysis+alcohol precipitation &pectinwashing pH adjust beforealcohol															
Varu *et al*.,2016	Kagzi lime peel 70ºC, 80ºC, 90 ºC g/mol	Gujarat								12.6-13.6, 11.67 - 12.07, 10.33-12	909.9 - 1020.41, 724.64-793.65 328.95-335.57	4.65, 6.63, 8.0	46-50, 70-76, 70-80	7.45 to 7.84, 8.16 to 9.39, 9.22 to 9.80		
Yadav,2017	Sweet lime pH 1,2,3	Allahabad	Brown	Dissolved slightly after vigorous shaking	Dissolves	Suspension form yellow ppt	Suspension dissolves & turn milky white			40,23, 27	694.44, 657.39, 634.17	9.3, 8.9, 8.23	10, 9.1, 8.3	12.5, 10.8, 8.10	78.14, 69.38, 67.76	67.57, 63.15, 57.17

Table 2.5: Review on functional group present in pectin of different citrus germplasm

Sl. No.	Functional group	Bond	Kanmani *et al*.,2014 Frequency (cm $^{-1}$)	Ahmed and Sikder, 2019 (Ginger lemon, Cardamom lemon, China lemon pectin) Frequency (cm $^{-1}$)
1	Alcohols, phenols	O-H stretch, H-bonded	3595.31 (s, sh)	3142.04 to 3560.56,3159.40 to 3300.20,3277.06 to 3462.22
2	Alkanes	C-H stretch	2931.80 (m)	2947.43,2941.44,2952.37
3	Alkanes	C-H stretch	2862.36 (m)	-
4	Alkynes	C = C stretch	2222.00 (w)	-
5	unsaturated ester	C=O stretch	1728.22 (s)	1712.79,1707,1732.08 from methyl esterified carboxyl groups
6	Alcohols, carboxylic acid, esters	C-O stretch	1319.31 (s)	
		C=O stretch from free carboxyl groups		1640.23,1622.13,1602.85
7	Aliphatic amines	C-N stretch	1242.16 (s)	-
8	Alkyl halides	C-H wag (-CH_2X)	1149.57 (m)	-
9	Aliphatic amines	C-N stretch	1095.57 (m)	-
10	Aliphatic amines	C-N stretch	1056.99 (m)	-
11	Aliphatic amines	C-N stretch	1026.13 (m)	-
12	Alkyl halides	C-Cl stretch	804.97 (m)	-
13	Alkyl halides	C-Cl stretch	840.98 (m)	-

2.8. Fruit peel and pomace as animal feed

The citrus pulp can be used as a cereal substitute because of high net energy (1.66-1.76 Mcal/kg DM) for lactating cows. Further 50 % of it can be utilized in the diet of gestating and lactating cows; 20-30 % in diet of rabbits and 5-10 % in poultry diets. Citrus pulp in the ratio of 70:30 produces excellent silage when combined with wheat or rice straw. They are rich bio active compounds which can be used for food and pharmaceutical applications (Wadhwa and Bakshi, 2013)

The citrus pulp can also be used as a cereal substitute due to its high organic matter digestibility (85-90 %) in concentrate diets and energy availability (2.76-2.9 Mcal ME/kg DM and 1.66-1.76 Mcal NE/kg DM) for the lactating dairy cows.

2.9. Mineral composition

The mineral composition of fruits largely depends on many factors such as soil type, stage of maturity, variety of cultivars, topography, and other geographical factors. Calcium is a structural component of bones and teeth, it plays role in cellular processes, muscle contraction, blood clotting, and enzyme activation (Gropper *et al*., 2005). 99 % of the calcium is found in bone and teeth in the body. Whereas the remaining 1 % is found in the blood and soft tissue. It is also required for stabilizing a number of proteins and enzymes, optimizing their activities (Weaver and Heaney, 1999).

Magnesium has a vital role in varying range of biochemical and physiological processes (Schrauzer, 2000). 60 % of magnesium is found in skeleton, 27 % in muscle, 6-7 % in other cells and < 1 % is found outside of cells (Shils, 1996). It is also being involved in more than 300 essential metabolic reactions (Spencer *et al*., 1994). It is also required for active transport of ions i.e potassium and calcium across the cell membranes (Rude and Shils, 2006).

As illustrated by Knochel, 2006 phosphorus is required by every cell in the body for maintaining normal function. In the body phosphorus is found as phosphate. 85% of phosphorus in the body is found in bone. It is a major component of bone in the form of calcium phosphate salt i.e. hydroxyapatite. Likewise, phospholipid is a major components of cell membrane. Moreover, ATP and creatine phosphate helps in energy production and storage. It is also required for acid base balance by acting as buffer in the body (Knochel, 2006).

Sodium is the major cation in extracellular fluid and a key factor for maintaining body fluid. Phosphorus is actively involved in several biologically important compounds such as roles in bone mineralization, energy transfer and storage, nucleic acid formation, cell membrane structure, and acid–base balance (Gropper *et al*., 2005).

Potassium is recognized for its function in fluid balance and nerve impulse transmission. Higher potassium and lower sodium intake prevent hypertension, the cerebral vascular damages and the heart diseases (Cook *et al*.,2009). It is required for pyruvate kinase which is an important enzyme in carbohydrate metabolism (Sheng, 2000).

Copper is one of the trace elements in human and animal body. It is a functional component for cupro enzyme i.e. cytochrome oxidase which is required for cellular energy production (Turnlund, 2006). The waste from citrus pomace is rich in 1-2 % calcium and 0.1 % phosphorus (Crawshaw, 2004; Bakshi and Wadhwa, 2013.

The key trace elements like zinc, selenium and iron are getting attention due to antioxidant defense mechanism. In addition, iron, copper, zinc and selenium are required to maintain stability and nutrion for human and animal (Rojas *et al*., 1999).

Nowadays nickel and arsenic are also gaining attention for animal and human nutrition. Zinc has also been used as cellular antioxidant (Powell, 2000).

As reported by Wood and Ronnenberg, 2006 iron is an element for metabolism of all living organism. It is also an essential component of hundreds of protein and enzymes.

Zinc is an important element which is required for cellular metabolism. It plays an important role in growth, development, immune response, neurological function and reproduction. It plays an important role in the structure of proteins and cell membranes (Cousins, 2006)

Silicon is important in fixing of calcium; treatment of osteoporosis (Jyotsna and Saonere, 2011). Daily recommended dose of magnesium is 150 to 500 mg of magnesium for the adults (Ilja and Hollman, 2005) as per the European health authorities' recommendation. Thus, the peels can be consumed as food additives in order to fill in the magnesium deficiencies from the children and old people in the rural zones of the developing countries

Table 2.6. Review on mineral composition in different citrus germplasm

References	Fruit	Place	Elements																								
			Ca	Cu	Fe	K	Mg	Mn	Na	Zn	Al	Pb	Ag	Cd	Ba	Si	Ni	P	B	Mo	Li	S	I	Co	Hg	As	Cs
Ali *et al.*,2016 (ppm)	Sour orange , Sweet orange, Grape fruit, Lemon	Pakistan	3700	ND	38.013	165750	530	4.5	21750	3.5	6.5	ND	ND	ND	ND	ND	ND							3.13 3.42 2.23 4.31 5.19 3.49 1.97 2.45 2.91 3.14 3.01			
			5970	ND	33	71000	462.0	5.5	6000	3.5	39.5	2	4	ND	ND	ND	ND										
			11623	ND	5	200	1508	3.5	1000	5	ND	1	3	ND	ND	ND	ND										
			6919	ND	12	218000	787	3.0	8000	10	7	3		ND	ND	ND	ND										
Ahamad, 2015 (ppm)	*C sinensis*	Saudi Arabia	91.2		25.34	829.3	28.3	0.25	138.2	0.58						195.3	1.08	130.9	125.6		1.91	2184.9	0.58				
Assa *et al.*, 2013 (mg/100g)	*Citrus sinensis peel* (green and yellow color) mg/100 g	Yopougon, Abidjan,	470.5, 490.5			1565, 1490	62.98, 41.83									10.31, 12.39		43.34, 36.33				10.69, 19.43					
Barros *et al.*,201	Lima orange, Pera	Brazil	18.7 ,20.3 ,0.2 , tr, tr	46.8 ,35.6 ,	171.0, 25, 6.4,16	140.1 , 139.0 , 168.8,1	10.3 , 10.3,8.5 ,	45.4,53.8 ,36.7,33	2.0 ,2.6 2.8 ,1.	91.3 ,67.2 ,																	

2 (mg/ 100g)	orange Tahiti lime, Sweet lime Ponkan mandari n pulp		16.0	69.0 , 25.3 , 48.9	9.1, 106.8 ,156.5	01.2 , 140.5	4.8 ,9. 4	.8 , 46.3	6 , 2.9	93.6 , 61.7 69.5																	
	Lima orange, Pera orange Tahiti lime, Sweet lime, Ponkan mandari n peel		145.2,1 65.4 ,2 14.2 ,1 21.2 , 85.5	58.6, 88.3, 57.6, 72.7, 55.0	1008. 6, 731.0, 768.7 ,943.4 , 321.5	258.7,26 6.0 187.1,14 2.1 , 151.0	23.8 , 27.8 , 41.2,1 9.7,13 .4	339.5, 212.1 183.7 ,200.3 , 95.3	85.1,6 0.1,54 .0,37. 7 ,32. 4	352. 0,21 4.1 200. 3,15 1.4,1 11.2																	
Boudrie s*et al.,* 2012 (mg/g)	Mandarin, Rocomora , Merme, Cherylan d, pulp	Algeri a	0.39,0.2 6,0.42,0. 30, 1.46, 0.28,0.2 9	0.04,0 .01,0. 01,0, 0.01, 0.03, 0.03	2.07,1 .92,2. 43, 2.01,2. 77, 2.64,2. 60	4.27,5.1 6, 5.22,5.0 8, 5.31,6. 00, 5.43	1.07,0 .6,0.6 6, 1.69,0 .8,0.5 2, 1.63	0.05,0. 05,0.34 ,0.34,0. 53,0.45 ,0.47	1.80,1. 38, 3.43,2. 46, 3.4,3.4 1, 3.0	0.34, 0.37, 0.32, 0.22, 0.63, 0.19 0.31												2.85 , 5.07 , 3.42 , 2.40 , 3.14 , 6.95 , 2.73 , 2.1 2,6 .97 ,3, 2.9 9					

Chuku and Chinaka, 2014 (ppm)	Nigeria (ppm)	Grape, lime orange, lemon fruit juices	1.62,2.29,2.24,3.52			24.50,31.67 47.84 195.45	0.19, 0.36, 0.43 0.26																0.80, 2.99, 0.46, 1.33				
Czech *et al.*,2019 (mg/100g)	Orange, Pomelo Mandarin, LemonKey lime,Red grapefruit Green grapefruit, White grapefruit (pulp)	Poland	279,145, 249,180, 413,213, 245,226	0.6 ,0.5 0.4,0.40.4,0.3,0.5 ,0.5	3.7,4.6 2.4,2.8, 2.1,1.9, 2.1,2.0	1390, 1040,1330, 1130, 1450,1110, 1230, 1170	103,194 104,84 116,80.7,79.9 90.0	0.2,0.1 0.2,0.6 0.1,0.1 0.1,0.2	1.2,1.0 11.1 18.9 31.0 , 1.2, 2.1,1.6	1.7, 1.0, 2.3, 1.7, 2.4, 1.7, 2.2, 1.9								233, 189 187, 180 179, 151 190, 170									
	Orange ,Pomelo Mandarin ,LemonKey lime,Red grapefruit Greengrape fruit,White grapefruit (peel)ppm		419,288, 371,318 639,360, 389, 348	1.5,2.1 0.5,0.40.6, 0.8 0.8 ,0.7	5.1,5.2, 3.3,3.4, 4.1,2.3, 2.7,2.8	1540,1270,1410, 1270, 1520,1320,1330, 1290	132, 230 129, 115 130,10095, 111	1.3, 1.5 1.2,0.5 0.4,1.0, 1.0 0.7	5.4,6.8 10.9,19.9, 38.8,2.5, 3.1,2.5	2.5,1.22.9,2.82.6,3.33.0, 2.8								253,219 199,239 201, 200, 225,190									
Ghani *et al* 2017 (mg/kg)	*Citrus sinensis Mg/kg*	Sargodha five tehsil		4.04		173.42	71.67			66.27		1.68															

Hong *et al.*,2018 (mg/kg)	CheonhyehyangHallabongJinjihyangHwanggeumhyangRedhyang Kumquat Lime Lemon Yuzu Orange Grapefruit mg/kg	Korea	10.57,15.35,12.40,10.68, 13.78,53.88, 24.95,27.93, 75.29,37.16, 32.83	185.9, 363.0, 233.1, 239.0, 154.5, 315.3, 395.4, 405.8, 569.8, 581, 625.5μg/kg	0.18,0.23 0.12,0.16 0.26,0.28 0.17,0.31 0.33,2.51, 0.31	95.22, 182.3, 135.1, 95.13, 103.8, 128.3 270.4, 196.1, 197.4, 147.4, 188.4	9.24, 12.85 8.76, 7.50, 8.21, 20.99 10.82, 8.81, 26.42, 11.37, 12.27	668.5, 878.7, 668.2, 588.6, 907.2, 1902, 200.5, 35.35, 733.0, 228.1, 172.1 μg/kg	2.49, 5.25 2.56, 2.74 3.77, 7.07 4.51, 5.32, 10.62 2.26, 2.47	636.6, 1019, 726.4, 466.5, 615.8, 1611, 984.8, 764.1, 1608, 739.5, 1004 μg/kg	0.09 0.18 0.09 0.06 0.10 0.04 0.24 0.09 0.59 0.14 0.13	1.72, 3.74, 5.73, 2.89, 1.22, 1.69, 2.58, 4.98, 0.63, 2.60, 6.86,		0.12, 0.17, 0.12, 0.16, 0.11, 0.16, 0.19, 0.43, 0.12, 0.29, 0.33	103.51,76.39, 107.6 65.59 179.91,BDL, 86.52 106.37 BDL, BDL, 125.96 μg/kg		54.89 58.67 38.059 2.4880.41 58.7 47.52 46.7 556.255 2.05 77.65 μg/kg	13.2722.0913.419.27,13.02 15.9619.0218.0847.30 23.0425.13			0.03, 0.25, 0.10, 0.18, 0.12, 0.39, 0.63, 1.21, BDL, 2.62, 3.16				0.52 0.74 0.36 0.84 0.66 0.56 0.48 0.44 0.82 0.86 0.72	0.79 0.86 0.71 1.00 0.68 0.81 0.56 0.77 0.83 1.63 0.49	0.50, 0.67, 0.38, 0.52, 0.48, 0.07, 5.83, 4.13, 1.29, 0.96, 1.44
Janati *et al.*,2012 (mg/100 g)	Lemon (*C limon*)	Iran	8452.50	4.94	147.65	8600	1429.50		755.50	.94								6656.25									
Mbong *et al.*,2014 (mg/kg)	*Citrus reticulata*	Urban and rural area			332,352			22.7,18.1		16.4, 5.4		2.6,0.8		2.6,1.0													
Mohammed *et al.*, 2013 (ppm)	*Citrus jambhiri peel*	Sudan ppm	1030	0.24	4.49	1440	20.80	0.80	11.48	1.14		Nd	0.003	Nd			0.07							0.01			

Narjis *et al.*,2009	Orange peel	Baghdad		1.3	125			88		13		0.25		0.11			1.6	0.20									
Nesrine *et al.*, 2012 (mg/100 g)	Thompson, mandarin, lemon peel	Tunisia	485.73, 514.82, 747.36	1.58, 0.40, 1.19	7.96, 9.48, 7.87	816.24, 828.35, 527.92	111.09, 98.48, 182.08		148.28, 223.38,188	1.43, 5.48, 8.61																	
Olivia and Valdes, 2003 (mg/ Litre)	Seville orange (*Citrus aurantium*) Pulp	Spain		1.83				5.07		11.40	20.14	1.25		0.33	3.91		0.59										
	Seville orange (*C aurantium*) Peel	Spain		4.58				3.00		40.85	14.38	0.33		0.42	5.83		1.33										
Omoregha *et al.,* 2018 (mg/ 100g)	*Citrus sinensis, Citrus tangerine*	Nigeria	42,36		0.2,0.2	188,161	11,14		1.01,1.2	0.07, 0.3								16,12									
Osarumwense *et al.*, 2013 (mg/ litre)	*Citrus sinensis* peel	Nigeria		0.01			15.55	0.04		14				<0.0011			<0.05										
Ozcan *et al.*,2016 (mg/kg)	Portugal Lemon, Lime Orange, Grape fruit,oran	Portugal	9686.92 8227.78 10887 8135.2,	225.05 4.57 2.61 7.74, 1.94	17.95 22.93 20.42 18.35, 12.98 10.21,	9387.99 13973.70 9632.69	1796.41 1648.71 874.08 1491.5	1.47 157.67 283.14 264.8	801.22, 253.20, 282.11	6.45, 6.51 3.30 4.31, 4.10, 3.54,								1043.19, 864.04 1086.55	12.18 19.93 24.18	0.19, 0.15, 0.25,							

	ge (Laranja Nacional), Mandarin Spain Lemon, grapefruit , mandarin , orange Turkey fruit Orange, lemon, grape fruit, mandarin peel		9598.54, 6646.28, 10401.3 7, 8535.35, 7758.17, 6371.59, 5643.53, 10773.5 1, 9173.94, 6113.94	1.65, 2.56, 1.54, 1.86, 0.88, 1.75, 1.61, 2.43, 0.43	11.27, 11.81, 13.94, 11.32, 20.30, 19.35, 18.82, 12.82	6685.63, 7447.75, 12694.9 0, 11998.8 2, 8406.99, 9588.41, 14760.7 0, 16459.6 6, 5534.64, 6437.12, 5457.1 2	2, 2084.7 8, 1500.0 6, 1882.9 6, 1272.9 3, 1095.0 7, 940.29, 632.15, 1026.6 2, 860.84, 560.8 4	1, 166.4 4, 225.1 4, 90.08, 88.80, 87.82 2.26, 3.45, 9.03, 1.68, 1.08	522.6 0, 279.2 9, 474.8 7, 270.0 6,356. 49, 534.9 1, 313.6 6, 550.7 4, 256.6 5, 798.4 0, 738.4 0	4.37, 4.49, 3.39, 2.70, 6.27, 5.48, 4.43, 2.43								872.6 1, 418.9 9 487.8 0, 633.2 8, 685.1 7 531.8 6, 559.0 5, 832.3 3, 574.7 9, 687.9 6, 786.9 6	36.2 4, 38.3 5, 26.5 5, 10.5 5, 37.2 7, 36.5 4, 34.1 0, 43.9 1, 35.1 6, 31.4 5, 21.4 5	0.0 , 0.0 0.18 , 0.28 , 0.1 1, 0.02 0.0 , 0.11 , 0.03 , 0.23 , 0.1 3							
	Portugal Lemon, Lime Orange, Grape fruit, Laranjan acional (Orange), mandari n Spain fruit (lemon, grape fruit,		3473.1 2, 3336.85, 3091.8 3, 2419.8 6, 2703.3 3 2508.3 0, 3414.3 4, 3607.2 3 3156.2 0,	11. 21 4.4 4 2.6 0 2.8 8 1.9 9 2.1 5 2.4 4 1.7 5 3.0	13.3 2 15.6 9 9.63 9.47 12.1 7 8.10 10.0 3 27.4 7 10.3 9 10.6 0	15704.9 5 14061.7 2 10626.0 11335.5 3 9618.96 11724.4 2 11764.8 9 10946.1 8 16312.3 5 13570.7	1364.5 5 1650.14 868.02 1104.86 926.46 1283.13 1024.76 939.32 1353.76 1019.68 1159.02 1239.45 1061.92 1069.69	1.13 1.48 2.21 3.63 3.53 2.16 1.48 6.72 10.64 2.32 4.30 1.79 4.42 3.36	660.50 267.03 278.96 352.27 260.55 363.93 257.02 275.46 369.09 237.38 359.83 253.12 524.27 261.64	7.75 7.56 3.14 2.77 3.73 3.05 4.39 3.84 4.47 4.12 4.21 3.61 3.54 3.55								1605 7 1557 1 1557 3 1292 7 884.6 865.2 1074 1 984.9 1227 9 961.8 1111	.5 61.8 5 .847.5 3 .039.4 1 .123.3 9 328.9 47 .326.4 9 623.5 .33 20.5 31 .937.1	0.13 0.08 0.03 0.00 0.07 0.37 0.01 0.02 0.17 0.16 0.15 0.19 0.13 0.18							

	mandari n, orange), Turkey fruit Orange, lemon, grape fruit, mandarin Mandari n (pomac e)		2234.7 2, 3134.1 0, 5312.3 1, 2669.9 2 4564.4 8	3 1.3 1 3.2 7 2.4 3 4.3 1 2.66	12.3 5 9.24 12.0 2 9.22	6 11672.4 9 12218.0 9 8682.77 10478.1 9												3 1093 9 1048 7 1518.3 1	2 .214.2 8 .221.5 8 25.2 9 19.0 9 15.1 0								
Paul and Shaha, 2004 (mg/ 100g)		Ban glad esh	255,70 0, 200,100	2,2.6 0.6,1 .9	3.8,2.3 5 ,4	994,148 0 2000, 1060	162,1 20 100,2 16		280,1 5 10,27	4.8, 1.2 1.4, 1.5								242, 100 200, 200									
Peace and Happiness, 2017 (mg/100g)	*Citrus maxima* juice and peel extract (mg/ 100g)	Nige ria	132.76 ,515.78		2.17 , 9.06	1.3,8.7 5	1.88,5. 39		46.12, 274.7 7									38.9 6 366.8 4									
Rangel *et al*.,2011 ((µg/g)	Acid lime fruits (*Citrus la tifolia*) conventi onal& biodyna mic juice µg/g	Brazil	23.24, 23.41	0.35, 0.20	1.71,0 .75	376.79, 240.70		0.08,0. 04		0.29, 0.43																	
Roghini and Vijayalaks	*Citrus paradisi*	Tam il Nad			51.2		345	49.5		98.5																	

h mi ,2018 (ppm)		u																									
Singh & Raj, 2018 (mg/100 g)	*Citrus limon* peel	Luck now, Indi a	134		0.80	160	15		6									12									
Uraku,2015 (mg/ kg)	*Citrus sinensis Vitis vinifera*	Nigeri a	151, 8.45			204.33, 280.05	18.33, 95.48		2.67, 12.47																		
Zaker,2017 (mg/100 g)	*Citrus sinensis* Peel& pomace	Parbh ani	501,27 4		7.1,11 .6	1109,1 191	116,1 14											203, 273									

Chapter 3

Materials and Methods

The present experimental finding **"Essential oil, pectin and ionome profiling of Citrus fruit waste"** was conducted during the year 2016-2018 at the Department of Horticulture, Sikkim University, Tadong. The materials utilized, the experimental approaches and the procedures adopted during the course of investigation are described below.

3.1. Material:

3.1.1. Collection of samples

Citrus germplasm was collected from the month of August to December 2016. The collections were made from the private holdings and government owned farm *viz*: Horticulture farm of state government, Indian Council of Agriculture Research (ICAR) farm and Krishi Vigyan Kendra (KVK) of Sikkim representing different altitudes of Sikkim. The GPS coordinate were taken for the collected samples.

Citrus germplasms *viz:* Sikkim Mandarin (*Citrus reticulata* Blanco), Pommelo (*Citrus maxima* Merr.) and Rough Lemon (*Citrus jambhiri* Lush) were taken for the present investigation which were the experimental materials taken in the present study which were collected from five different altitudinal range *viz:* 800-1000 m, 1000-1200 m, 1200-1400 m, 1400-1600 m and >1600 m from Mean Sea Level (MSL.). The samples were then placed in separate container to get rid of moisture loss during its transportation to laboratory for further analysis.

The collection site, their respective GPS data and details of germplasms are illustrated in Table No 4.1.2. Fruits were collected in two stages i.e. green mature stage and ripened stage.

Table 3.1. List of Germplasm considered for the study

Sl. No.	Common Name	Scientitifc Name
1	Sikkim mandarin (G_1)	*Citrus reticulata* L.
2	Pommelo (G_2)	*Citrus maxima* Merr
3	Rough lemon (G_3)	*Citrus jambhiri* Lush

3.1.2. Sample Preparations

The fruits were tagged separately and were washed, demucilaged to remove peel and pomace after juice extraction. The peels and pomace were then kept in a separate folded paper bag and dried in a hot air oven for 72 hours with the details. After drying, the samples were grounded to fine powder with the help of grinding mill and passed through 24 mesh sieves. The dried powder was used for proximate analysis.

On the other hand, samples required for ionome profiling were washed with double distilled water, cut into pieices after extracting seed and finally kept in a paper bag with proper labeling which was then placed inside the hot air over at around 60°C for 48 hrs. After drying, the samples were then made into fine powder using grinding machine.

The fine grounded powder was stored in air tight plant sample container for nutrient analysis. The fresh peel was taken for extracting essential oil. All the analysis was performed using analytical grading reagent in triplicate.

3.2. Proximate analysis

3.2.1. Physico chemical characterization

Citrus fruits of different cultivars were analysed for various physicochemical characteristics *viz.* weight, length, diameter, volume, moisture, ash, crude fibre, TSS, titrable acidity, TSS/acidity, ascorbic acid, reducing sugar, total sugar, crude protein, total phenol and antioxidant scavenging activity (DPPH).

3.2.2. Weight of fruit, peel and pomace

The weight of the fruit, peel and pomace was measured using digital weighing balance and expressed as gram (g). Sample dry weight was taken after the drying the peel in hot air oven for 72 hours at 60°C followed by grinding using Willy's Mill Grinder (Space-N- Service), India. The juice was extracted from the cleaned fruits using juice extractor (Gryphon, China) and the resultant pomace was dried at 60° C for 72 hours in the hot air oven followed by grinding into powder by using Willy's Mill Grinder (Space-N- Service), India.The powder were sieved (Sieve No MIC - 300) and packed in container. The obtained powder was stored at 6-10 ° C for further analysis. The dried pomace was then measured to know the difference in moisture content.

3.2.3. Diameter and length

Diameter and length of the fruit samples was determined with the help of vernier callipers and expressed as centimetre (cm).

3.2.4. Moisture content

The moisture content was determined by gravimetric method. 2 g sample was placed on pre-dried and cleaned crucible. It was then placed at hot air oven at 100 ^{0}C. There after the crucible was removed, cooled in desiccators and weighed.

The moisture was calculated by using the following formula:

$$\text{Moisture content \%} = \frac{\text{Weight of the residue}}{\text{Weight of the sample}} \text{ X } 100$$

3.2.5. Total Soluble Solids

The juice of fruit samples was used for analysis of TSS in the hand operated refractrometer. The refractometer was calibrated with distilled water before use.

3.2.6. Titrable acidity

Titrable acidity was estimated by method suggested by AOAC, 2000. Fruit pulp was mixed with the help of mixer thoroughly. Juice was separated through filtration with the help of muslin cloth.

10 ml of well mixed juice was taken which was diluted to 250 ml with neutralised or recently boiled water. Then titration was done with 0.1 N NaOH using 1 % phenolphthalein solutions as indicator. The titre values were recorded when the solution turns pink in colour. The % titrable acidity was expressed as percent citric acid equivalent using the formula.

$$\text{\% titrable acidity} = \frac{\text{titre X normality of the alkali x vol. made up x equivalent wt. of acid X 100}}{\text{volume of sample taken for estimation x wt. or vol. of sample taken X 1000}}$$

3.2.7. TSS: acidity ratio

It was calculated by dividing TSS from titrable acidity.

3.2.8. Refractive Index (RI)

The Refractive Index of essential oil of peel sample was estimated using refractometer. Few drops of essential oil were placed between the prisms of refractometer. The telescope was then rotated to bring the border line of total refraction to the junction of cross-wire in the telescope. The refractive index was recorded.

3.2.9. Vitamin C

Vitamin C content was determined as per AOAC, 2000. Ascorbic acid standard was prepared using 3% metaphosphoric acid as a solvent as recommended by AOAC, 1968. The dye solution was prepared with 50 mg of sodium salt of 2, 6 dichlorophenol-indophenol dissolved in 150ml of hot distilled water to 200 ml.

For ascorbic acid estimation, standardization of dye was done by mixing 5ml of standard ascorbic acid solution with 5ml HPO_3. The burette was then filled with the dye solution and titration was done with ascorbic acid mixture until the appearance of pink colour which persists for 15 second. Dye factor was calculated by the formula

$$\text{Dye factor} = \frac{0.5}{\text{Titre}}$$

10 ml of juice sample was extracted and volume made up to 100ml with 3% metaphosphoric acid. From the prepared juice sample, 5ml of juice was taken and titrate with the standard dye to a pink end point which should persist for at least 15 sec. Vitamin C content of sample was calculated by using the following formula.

Calculation

$$\text{mg of ascorbic acid/100g} = \frac{\text{Titre X dye factor X vol. made up X 100}}{\text{Aliquot of extract taken X wt or vol. of sample taken for estimation}}$$

3.2.10. Reducing sugar

The estimation of reducing sugar was done by applying fehling solution A and B as suggested by (Lane and Eynon, 1923). 10 ml of sample fruit juice was taken in 500 ml volumetric flask and some amount of water was added. 0.1 N NaOH was added to neutralize the mixture followed by few drops of phenolphthalein, which was transferred to 250 ml conical flask. In that 25 ml of 45 % neutral lead acetate solution was added, shaken and let stand for 10 min. After 10-minute 25 ml of 22 % potassium oxalate solution was added. The final volume was made to 250 ml which was then filtered. The filtrate was then transferred to a 50 ml burette having an off-set tip. 5 ml each of Fehling A and B solutions was taken into 250 ml conical flask few drop of methylene blue indicator was added to the boiling Fehling solution. Then the sugar solution was dispense from the burette to the conical flask until the blue colour disappears to a brick-red end point. The titre value was recorded from the burette. The titration was performed in triplicate and average was taken.

Formula for calculation

$$\text{reducing sugar (\%)} = \frac{\text{Fehling's Factor X dilution}}{\text{titre X intial weight of sample}} \text{ X 100}$$

3.2.11. Total sugar

Total sugar was also determined by (Lane and Eynon,1923) method using Fehling solution A and B after hydrolizing non reducing sugar with dilute acids to reducing sugar. The remaining 100 ml of unhydrolised deleaded filtrate sample solution after estimation of reducing sugar was added into 250 ml of volumetric flask. In that 20 ml of 50 % HCl was added and kept overnight for hydrolization. The very next day few drops of phenolphthalein indicator were added and then conc. NaOH also applied drop by drop until light pink colour appeared. Once the pink colour developed, few drops of conc. HCl was added till disappearance of pink colour. After that final volume was made to 250 ml with distilled water, if the turgidity still persists then the filtrate solution was further filtered using filter paper.

The solution was then transferred to a burette for titration. 5 ml each of Fehling solution A and B was taken in a conical flask and heated to boil. While boiling few drops of methylene blue indicator was added. Then the filtrate containing sugar solution was dispense from the burette to the conical flask until the blue colour disappeared and attained brick-red end point. The titre value was recorded from the burette. The titration was performed in triplicate and average was taken.

Formula for calculation

$$\text{Total sugar (\%)} = \frac{\text{Fehling's factor X dilution}}{\text{titre X intial weight of sample}} \text{ X } 100$$

3.2.12. Non reducing sugar

The non reducing sugar was calculated by

Non reducing sugar = (total sugar-reducing sugar) X 0.95

3.2.13. Crude fat

The crude fat content was analyzed by essential oil extractor; model no Socsplus-SCS 06 DLS, PELICAN, India by following solvent extraction method (AOAC, 2000). The estimation was done by placing powdered sample (2 g) marked as W into the thimble. Then the weight of empty beaker was taken as W_1. Now, once the sample was placed in the thimble it was inserted into the beaker by fixing it with thimble holder. The beaker was then filled with methanol (70 ml) and then the beaker along with solvent was fixed into the extraction system and water tap was open for condenser.

At the intial stage, the machine temperature was adjusted to 110° for 45 minutes to operate boiling and the stopper was closed during this stage to avoid evaporation of the methanol (solvent). Once the boiling is completed then the temperature was increased to 160° C for about 90 minutes for the evaporation of the solvent. As soon as the solvent along with samples starts drying in the beaker, the remaining solvent left in the system was collected. The beaker and the thimble were taken out from extraction system. Now the beaker was taken out to take the weight which was marked as final weight (W_2) and the estimation of crude fat content was done by following calculation.

$$\% \text{ Crude fat content} = \frac{W_2 - W_1}{W} \times 100$$

3.2.14. Crude fibre

The estimation of crude fiber content was done in fibre extraction machine (model No Fibra plus-FES 04 AS DLS, PELICAN India) as mentioned by Sadasivam and Manikam, 2000. In the empty crucible (W_1), 2 gm of the dry sample was taken (W). Then the crucible was attached to the extraction machine with the tap water opened through out. Now, 1.25 % H_2SO_4 (100-150 ml) was transferred to the machine from the top. At the intial phase i.e. boiling, tempertature and time was adjusted to 500 º C for 30 min. Then once the extraction was done, all the reagent was drained out from the fibra flow. The final phase is called digestion which was intiated by pouring 1.25

% NaOH (150 ml) from the top for acid digested sample. In this stage temperature and time were 400º C for 45 minutes. Once digestion was completed, the reagent was drained out as earlier. Distilled water was used for clearing the stocked sample/particles in the glass container. The crucible with the sample was then taken out from the extraction machine and was transferred in the hot air oven (70 º C) for drying.

The dried sample with crucible was then transferred into muffle furnance at 550°C for 3 hrs. then it was cool down and weighed (W_2).

Crude fibre content of the sample was calculated by following formula:

$$\text{Crude fiber content (\%)} = \frac{W_1 - W_2}{W} \text{X100}$$

W_1= Initial weight of crucible

W_2= Final weight of crucible with sample

W= Weight of sample

3.2.15. Ash content

Sample (2g) after crude fibre extraction was weighed and place into tarred silica crucible. The sample was burnt in muffle furnace 600°C for 6 hours. Crucible with burnt samples was transferred to desiccators and cooled to a room temperature. When cooled the crucible was weighed as quickly as possible to avoid moisture absorption (AOAC,1990).

Calculation

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Weight of the sample taken}} \text{X} \quad 100$$

3.2.16. Crude protein

The micro-kjeldahl method is most reliable method for nitrogen estimation. The percentage of protein can be determined by first determining the nitrogen content and multiplying the percent nitrogen by the factor 6.25 (AOAC, 1970). Accurately 40-50mg of sample was weighed and placed in a digestion flask. 1 g of catalyst mixture (grind together of K_2SO_4, 4.1 g of HgO and 0.8 g $CuSO_4$) and 5ml of concentrated sulphuric acid was added. The sample was digested using digestion unit till the solution becomes colorless (approximately 40 to 60 minutes at 370ºC).

The sample was cooled and minimum quantity of water was added to dissolve the solid. Then the digest was transferred to a distillation apparatus. After that 100ml of conical flask containing 10 ml of boric acid was placed. Then 2-4 drops of mixed indicator dye (1 part of 0.2 % methyl red in ethanol with 5 parts of 0.2 % bromocresol green in ethanol) was added and the flask was placed beneath the condenser with the delivery tip immersed in the solution.

The digest was then transferred to distillation apparatus and to it 8 to 10ml of sodium hydroxide- sodium thiosulphate solution to the digest and steam distillation until about 20 ml of distillate was collected in the conical flask. The tip was then rinsed with tap water and titrate the distillate against the standard acid solution until the first appearance of violet colour as the end point. Blank was also run in the same manner containing the same quantities of all the reagents but without sample for every nitrogen determination process.

$$\% N = \frac{\text{ml HCl used in determination- ml HCl used in blank X Normality of HClX14X 100}}{\text{mg sample}}$$

% Protein= % N X 6.25

3.2.17. Starch

Estimation of starch was done with perchloric acid after removal of sugar by extracting the sample flour with hot 70 % alcohol. In hot acid medium starch get hydrolyzed to glucose and dehydrated to hydroxyl methyl furfural. This compound when react with anthrone, forms a green color. 100 mg of sample was extracted with hot 70 % ethanol. Then it was centrifuge and residue was retained. The residue was then washed with 70 % hot ethanol. 5ml of water and 6.5 ml of 52 % perchloric acid was added to the dried residue and shake well, for 5 minutes, centrifuged and supernatant was collected.

The extraction process was repeated using 5 ml of fresh perchloric acid for 10 minutes. The pellet was re extracted by centrifuging for 30 minutes using 5 ml of fresh perchloric acid. The supernatant was pooled, and volume was made to 100 ml with water. Suitable quantity of aliquot was taken for glucose estimation. Now 5 ml of anthrone reagent (200 mg of anthrone dissolved in 100 ml of ice-cold 95 % sulphuric acid) was added and was heated on hot water bath for 10 minutes. It was then cooled rapidly, and the intensity of color formed was read at 620 nm by using Perkin Elmer Lamb 35 UV/VIS spectrophotometer. The glucose content was determined by using standard curve. The value was multiplied by factor 0.9 as 0.9 g of starch yield 1 g of glucose on hydrolysis (Clegg, 1956).

3.2.18. Antioxidant activity

The dry powder sample (10 g) was transferred to a dark coloured flask and was dissolved in 200 ml of methanol (70 %) for 24 hours which was stored at room temperature. The extract was then filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents after 24 hours. The process was further repeated after 48 hours. The combined supernatants were evaporated to dryness under vacuum at 40ºC using rotary evaporator under reduced pressure and weighed. The obtained extracts were then kept in the sterile container by diluting with required quantity of methanol and stored at refrigerator (4ºC) for further analysis.

3.2.19. DPPH

0.004 % w/v DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of methanol (80%) in dark room. Then 400 µg/ml solutions were made by mixing sample extract (4 mg) in methanol (10 ml). Likewise control sample was made by mixing DPPH solution (3 ml) and in all the cases methanol is used as a blank (Stankovic, 2011).

Now, a blank solution without plant extract with all threagent was done (A_b) and then the different concentration of plant extract mixed in methanol solution (2 ml) and (3 ml) of DPPH solution were added in several test tubes and were placed in dark for 50 minutes. If there is discoloration of color from purple to yellow it will indicate more antioxidant activity of the sample. The sample were placed at UV- Vis spectrophotometer along with blank (methanol) in 517 nm absorbance.

The calculation for radical scavenging activity (DPPH method) was done by:

% radical scavenging activity (DPPH method) = $\frac{\{(Ab-As)\}}{Ab} x\,100$

Where, A_b = Absorbance of control

A_s= Absorbance of sample extract/ Standard

Then % radical scavenging activity (DPPH method) was plotted against the concentration and IC_{50} was calculated.

3.2.20. IC_{50}

0.04 w/v DPPH solution was prepared by mixing together methanol (100 ml and DPPH (4 mg) in a dark room. A standard was prepared by using ascorbic acid stock solution which involves mixing together 2 mg ascorbic acid with 2.5 ml distilled water thus making the concentration of the solution 0.8 mg/ ml. Then the different concentration of ascorbic acid i.e. 0.025 mg/ml, 0.050 mg/ml, 0.10 mg/ ml, 0.20 mg/ ml and 0.40 mg/ml was made by diluting the ascorbic acid solution.

Likewise, sample extract solution was prepared by mixing together 4 mg of sample extract and 10 ml of methanol which make the concentration of 0.40 mg/ml. The solution was then diluted for making desired concentration. In the similar way control was also made which includes addition of 3ml of DPPH solution and methanol.

Now, plant extract solution (2 ml) were taken separately in different test tubes of varied concentration in which 3 ml of methanolic solution of DPPH was placed in different test tubes. after that all the testtube solutions were kept in dark for about 50 minutes.

The appearance of yellow color from purple in the solution will indicate more of the antioxidant activity. Finally, the absorbance was taken at UV-Vis spectrophotometer at 517 nm. In the similar way absorbance was also taken for the blank solution which contain all the solution except the plant sample extract.

The % free radical scavenging activity (DPPH method) was estimated by the following equation.

$$\% \text{ free radical scavenging activity (DPPH method)} = \frac{A_b - A_s}{A_b} \times 100$$

Where, A_b = Absorbance of control

A_s= Absorbance of sample extract

After that the % free radical scavenging activity (DPPH method) was plotted with that of the different concentration in the graph from which IC_{50} was found.

3.2.21. Total Phenol

An estimation of total phenol was using Folin-Ciocalteu reagent (FCR) as per Standard method of biochemical analysis (Thimmaiah, 1999). 0.5 g of sample grounded with ten times the volume of 80 % ethanol. It was then centrifuge for 20 minutes at 10,000 rpm. The resultant supernatant was collected and the process was repeated five times. The collected supernatant was evaporated to dryness to which 5 ml of distilled water was added. Aliquot of 0.2 to 2 ml were pipette out in several test tubes.

The volume was made up to 3 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent was added. After 3 minutes, 2 ml of 20 % sodium carbonate was added in the same test tube. The solution was mixed together and boiled for one minute. After cooling, absorbance was read at 650 nm against reagent blank. A standard curve was prepared by using catechol of different concentrations. The phenol concentration was made from the standard curve and was expressed as mg phenols/100 g material.

3.2.22. Flavonoid

Total flavonoid contents were estimated by method described by Ordonez *et al.,* 2006. 0.5ml of extracts (1mg/ml) was mixed with 0.5 ml of aluminium chloride (prepared in 2 % ethanol). The resultant mixture was then incubated for 60min at room temperature for development of yellow colour which indicated the presence of flavonoid.

The absorbance was then measured at 420 nm using Perkin Elmer, Lamb 35 UV–VIS spectrophotometer. Total flavonoid content was calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve: Y = 0.217 x,

R^2 = 0.958, where X is the absorbance and Y is the quercetin equivalent.

3.3. Essential oil extraction and characterization

The peels of mandarin, pommelo and rough lemon were washed with water, cleaned and peeled. The peels were then weighed in digital weighing balance and transferred to a 2000 ml round bottom flask with volume of water enough to cover the peels. The flask was placed above the heating mantle and was connected to Clevenger and finally to a condenser with inlet connected to tap water and outlet for water circulation using silica pipe. Initially the temperature was maintained at 60º C and in some cases increased higher depending on the boiling requirement.

The process of boiling was continued for three hours. The distillate (a mixture of oil and water) so obtained was collect to separating funnel for separation of oil at the upper layer and water at the lower layer. Water discarded and the oil was collected in a culture tube, while any remaining water droplets in the oil were then removed by the addition of anhydrous sodium sulphate.

All the extracted essential oil from citrus germplasm were then subjected for profiling and characterization i.e. physical and chemical parameter following standard procedure and protocol as follows:

3.3.1. Physical parameters

3.3.1.1. Yield (%)

The yield of the extracted oil was calculated using equation

$$\% \text{ yield} = \frac{\text{Weight of oil extracted}}{\text{Weight of sample used}} \times 100$$

3.3.1.2. Determination of the solubility of citrus peel essential oil in water

A few drops of the oil were added to a test tube containing little amount of water stirred thoroughly with a stirring rod. Two separate phases were observed. The insolubility of the oil in water was inferred from that operation.

3.3.1.3. Specific gravity

Specific gravity was determined by drying the oil using anhydrous sodium sulphate and filters the oil through filter paper. The sample was cooled to 20-23° C and fills the pycnometer to overflowing. Care was taken to avoid air bubbles. Insert the stopper and incubate in a water bath at 25±0.2°C for 30 min.

Calculation

$$\text{Specific gravity at } 25° \text{ C} = \frac{\text{Weight of oil}}{\text{Weight of water}}$$

3.3.1.4. Color

Color was measured in a spectrophotometer using carbon tetrachloride as blank at the wavelength of maximum absorption.

3.3.1.5. Refractive index

The Butyro refractometer was standardized with distilled water, then it was cleaned with acetone and dried. Few drops of essential oil were then placed between the prisms of refractometer. The telescope was rotated to bring the border line of total refraction to the junction of cross-wire in the telescope. The refractive index was then recorded.

3.3.2. Chemical Characteristics

3.3.2.1. Saponification Value (S.V)

0.5 N alcoholic potassium hydroxide: 30 g of KOH was dissolved in 20 ml of distilled water and sufficient amount of aldehyde free alcohol was added to make 1000 ml. It was allowed to stand overnight, the clear solution was decant and stored in a bottle with rubber stopper. 1 ml of oil sample was taken in 250 ml conical flask and was kept above heating mantle.

In that 25 ml of alcoholic potassium hydroxide solution and few glass beads were kept. It was then connected to an air condenser (65 cm). It was boiled gently until the sample was completely saponified which was seen by the absence of any oily matter and appearance of clear solution. Both the flask and condenser were allowed to cool down. The inner side of condenser was washed with hot ethyl alcohol (10 ml) neutral to phenolphthalein. Now 1 ml of phenolphthalein was added to the flask and titration was done with standard HCl. Blank was also taken in a same way excluding only the oil sample.

Calculation

$$S.V = \frac{\text{Blank titre - Sample titre X N of KOH X 56.01}}{\text{Weight of sample (g)}}$$

3.3.2.2. Iodine Value (I.V)

Oil sample (0.5 ml) was poured into a glass stoppered conical flask and 10 ml of CCl_4 was added to it. The entire content was dissolved in 25 ml of Wij's solution by swirling. The Wij's solution was prepared by dissolving 8 g of iodine trichloride in 200 ml of glacial acetic acid and mixed with 9 g of iodine dissolved in 400 ml of glacial acetic acid.

The stopper was replaced after moistening with potassium iodide solution and was stored in a dark cupboard for 30 minutes. 10 % of potassium iodide solution was then prepared by dissolving 10 g iodine in 90 ml distilled water. Meanwhile 15 ml of 10 % KI solution and 100ml distilled water was added. It was then titrated with 0.1 N $Na_2S_2O_3$ using starch indicator near the end point. Blank determination was also done with the same procedure without the oil sample.

The iodine value was calculated using equation

$$I.V = \frac{\text{(Blank titre – Sample titre) X N of } Na_2S_2O_3}{\text{Weight of sample (g)}} \text{ X } 12.69$$

3.3.2.3. Thiocyanogen value (T.V)

Potassium iodide solution was made by dissolving 150 g KI in 1000 ml distilled water. Lead thiocyanate solution: 250 g of neutral lead acetate was dissolved in 500ml of distilled water and 250 ml of potassium thiocyanate in 500 ml distilled water. The two solutions were mixed by stirring continuously. It was filter using Buchner funnel and the precipitate was washed with water and ether. The precipitate was dried in a desiccator over P_2O_5 for 8-10 days.

0.2 N thiocyanogen solutions: 50 g of lead thiocyanate was dissolved in 500 ml of anhydrous acetic acid. In another beaker 5.1 ml bromine was mixed with 500 ml of glacial acetic acid. The bromine solution was then dispersed into lead thiocyanate solution until it become colorless. The precipitate of lead bromide was obtained and excess lead thiocyanate which once settled was filtered through Buchner funnel. It was re filter till clear solution was obtained. It was then stored in amber colored glass-Stoppard bottle at 18º-21º C.

1 ml of oil was mixed with 25 ml of thiocyanogen solutions and stored in a dark place at 18 to 21º c for 24 hours. Three blank determinations were done with sample. After 24 hours, the samples were taken out from dark and mixed thoroughly with 1.66 g of fry, powdered KI for 2 minutes. After mixing for three minutes, 30ml of distilled water was added. Now titration was done with 0.1 N $Na_2S_2O_3$ using starch indicator.

Calculation

$$T.\ V = \frac{(\text{Blank titre} - \text{Sample titre}) \times N \text{ of } Na_2S_2O_3 \times 12.69}{\text{Weight of sample (g)}}$$

3.3.2.4. Acid Value

Neutralized ethanol was prepared by boiling ethanol and titrating against 0.1 N NaOH using phenolphthalein as an indicator. 10 ml of oil was mixed with hot 100 ml of neutralized ethanol and titration was done using 0.1 N NaOH using phenolphthalein indicator. The solution was shaked vigorously during titration.

$$\text{Acid Value} = \frac{56.1 \text{ X Normality of NaOH X Titre value}}{\text{Weight of Sample (g)}}$$

3.3.2.5. Free Fatty acid

The free fatty acid was determined according to the method prescribed by F.S.S.A.I, 2012 using the acid value with the formula as:

Acid value= Free Fatty Acid (%) X 1.99

3.3.2.6. Peroxide value

The peroxide value of oil was determined using acetic acid-chloroform mixture, saturated KI solution, standard 0.01N Sodium thio sulphate solution and 1 % starch indicator as described by Ranganna, 1986.

Glacial acetic acid and chloroform were mixed together at 2:1 ratio to make the solvent. Then saturated KI solution was prepared by dissolving 4 part of KI in 3 parts of distilled water. The solution was then kept in a brown colored bottle. In a conical flask containing 1ml of oil, 25 ml of the solvent 1 ml of KI solution was added and shaken for 1 minutes. Then 35 ml of water was added and titrate the liberated iodine with 0.1 N $Na_2S_2O_3$ using starch indicator. Blank determination was also performed without oil sample.

Calculation

$$\frac{\{\text{Sample titre- Blank titre}\}\text{X Normality of sodium thiosulphate solutionX1000}}{\text{Weight of oil taken}}$$

3.3.2.7. Ester value

Ester value, which is defined as the number of milligrams of potassium hydroxide required to saponify the fatty acid esters in one gram of the oil, was also determined for the oil extracted in this work. It was obtained as the difference between the saponification value and the acid value of the oil (Saad, 2007) as given in equation.

Ester value = Saponification value - Acid value

3.3.2.8. Polyphenol content

The phenolic content in oil sample was determined using spectrophotometric method (Singleton *et al*., 1999) using Folin-Ciocalteu reagent and Gallic acid. It was expressed in mgg^{-1} GAE. 20 µL of essential oil was diluted in methanol (1:60). It was then mixed with1 mL of folin-ciocalteu's phenol reagent. Sodium carbonate (1ml) was applied to the mixture after 3 minute and its volume was made to 980 µL with distilled water. It was then placed in a dark room for 120 minutes after that wavelength of 725 nm was used to estimate the phenol content in UV-Vis spectrophotometer. Gallic acid standard was being used for calculating the gallic acid equivalents.

3.3.2.9. Antioxidant activity (DPPH method)

The estimation of DPPH was done by spectrophotometric methods. The methanolic solution of 50 µL essential oil was made by dissolving in 1000 µL methanol. Five different concentrations of this methanolic solution (50, 100, 125, 150, 200 µL) were placed in test tubes and adjusted to 300 µL with methanol and 2700 µL fresh methanolic solution of DPPH was then added. The absorbance was recorded at 517 nm using the equation as:

$$\% \text{ inhibition} = \frac{A_0 - At}{A_0} \times 100$$

A_0 = absorbance at t= 0 and At = absorbance at t=90 minutes

3.3.2.10. GCMS analysis

Profiling of essential oil was done using GCMS analysis.

3.3.2.10.1. Preparation of sample for GC-MS analysis

A sample of 1 µl was used in a split plot ratio of 100:1. The instrument operates by using ionization energy of 70 eV along with helium as a carrier gas which

The electron ionization with energy of 70eV along with chemical ionization with Helium as a carrier gas at a flow rate of 1.5 ml/min was used in the process. Mass scanning (MS) range was varied over 50-550m/z while injector and MS transfer line temperatures was set at 220 and 290°C, respectively.

3.3.2.10.1.2. Compounds Identification.

Identification of volatile compounds in the essential oil was done by using retention indices (RI) which helps in identifying the peaks of the compound with those of authentic compounds available in NIST 14 and Willey 8 library. Further identification was made by matching recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC-MS data systems and other published mass spectra [Adams, 2001]. Compounds were identified by using the library databases of NIST147.LIB, NIST27.LIB and WILEY7.LIB.

3.4. Analysis of pectin

A dry mass of 10 g peel powder left after oil extraction was subjected to extraction using 5 gram of citric acid in 250 mL of distilled water. The mixture was heated at 80°C in a water bath for 10 minutes. After cooling the solution, it is filtered using cloth filter and Whatman filter paper under vacuum. Ethanol (40 ml) was then added to the filtered solution to facilitate filtration of pectin. The solution was now subjected to centrifuge at 8000 rpm for 15 min at 100°C to separate jelly pectin. The jelly pectin so obtained was dried in a hot air oven (Khule *et al*., 2012).

Fruit
↓
Peel
↓
Essential oil extraction
↓
Remains of peel dried in tray drier at 55°C for 24 hrs.
↓
Powder (With the help of grinder)
↓
Water with peel powder (20:1)
↓
Boiled (At pH 2)
↓
Cooled (room temperature)
↓
Separated by filtration (twice)(With 2 fold muslin cloth)
↓
Added absolute alcohol (double volume)
(95 % alcohol)
↓
Separated the precipitate
(With 4-fold muslin cloth)
↓
Dried (Tray drier)
↓
Grind
↓
Pectin powder
Stored

Fig. 1 Flow diagram for extraction of pectin

3.4.1. Qualitative tests

3.4.1.1. Pectin colour

Dried pectin samples were observed visually and the colours of samples was noted down.

3.4.1.2. Solubility in hot and cold water (dry pectin)

0.03 g of pectin samples was taken in different conical flasks and mixed with 10 ml of 95% ethanol followed by 50 ml distilled water. The mixture shaken vigorously to obtained suspension which was heated at 85-95°C for 15 minutes using magnetic stirrer.

3.4.1.3. Solubility in hot and cold alkali (NaOH)

10 ml of 0.1N NaOH taken in a conical flask, 0.1g of dry pectin was added and was heated at 85-90°C for 10- 15 minutes using magnetic stirrer.

3.4.1.4. Sugar and organic acids

0.1g of pectin samples was taken in each 250 ml flask and 5 ml ethanol was added to moisten it and 100 ml water was poured rapidly, shaken well and allowed to stand for 10 minutes. The solution was added with 100 ml ethanol (95 %) containing 0.3 ml hydrochloric acid mixed and filtered using muslin cloth. The 2.5 ml of collected filtrate was taken in a conical flask and the liquid was evaporated on direct flame, residue was collected dried in an oven at 50°C for 2 hr.

3.4.2. Quantitative tests performed

Determinations of equivalent weight, methoxyl content, ash content and moisture content.

3.4.2.1. Pectin yield

Pectin yield was calculated as follows:

$$Y_{pec}\,(\%)\ \frac{P}{Bi} \times 100$$

Where, Y_{pec} is the yield of pectin in (%), P is the amount of extracted pectin in g and Bi is the initial weight of fruit peel powder

3.4.2.2. Ash content

The estimation of ash content was done by burning 1g of pectin sample inside the muffle furnace for 3-4 hours at 600°C. The crucible containing ash was cooled at room temperature in a desiccator and was weighed again to determine the alkalinity, the ash was then dissolved in 25 ml of 0.1 N HCl. It was heated gently to boiling and subsequently cool down. Titration was done with 0.1N NaOH using phenolphthalein indicator.

Calculation

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Weight of pectin}} \times 100$$

3.4.2.3. Equivalent weight (E.W)

Equivalent weight was determined as described by Ranganna's method (1986). In a 250 ml conical flask containing 0.1g of pectin sample, 5 ml of ethanol 1g Sodium chloride and 100ml distilled water added. Phenol red (6 drops) was used as an indicator. It was ensured that the pectin samples get dissolved without clumping and it was now titrated against 0.1N NaOH till pink colour appeared as the endpoint persisted for at least 30 seconds.

Calculation:

$$\text{Equivalent weight} = \frac{\text{Weight of pectin sample}}{\text{ml of Alkali X Normality of Alkali}} \times 1000$$

3.4.2.4. Methoxyl content (MeO)

Neutralized solution obtained after equivalent weight determination was used for MeO content. To the solution, 25 mL of 0.25 N NaOH was added. This mixture was stirred and allowed to stand for 25- 30 minutes at room temperature. After that

25ml of 0.25 N HCl was added and titration was done with 0.1N NaOH till pink colour appeared as the endpoint and persisted for at least 30 seconds.

Calculation:

$$\text{Methoxyl content \%} = \frac{\text{ml of Alkali X Normality of alkali X 3.1}}{\text{Weight of pectin sample}}$$

3.4.2.5. Degree of Esterification (DE)

The degree of esterification of pectin was measured on the basis of methoxyl and AUA content (Owens *et al*., 1952) and calculated by using following formula.

$$\% \text{ DE} = \frac{176 \text{ X MeO}}{31 \quad \% \text{ AUA}} \text{ X } 100$$

3.4.2.6. Anhydrouronic Acid Content (AUA)

AUA content of pectin was obtained from equivalent weight, methoxyl content and the alkalinity of the ash data (Ranganna, 1986) by following calculation.

$$\text{AUA} = \frac{176(\text{m.e. alkali for free acid} + \text{m.e. alkali for saponification} + \text{m.e. titrable ash}) \text{ X}100}{\text{Weight of sample (mg)}}$$

3.4.2.7. Spectral Analysis:

The functional group present in pectin from citrus fruit was analysed using FTIR spectrophotometer. Initially background measurements were performed by calibrating the instrument. After background subtraction pectin sample was placed in the sample holder which is made of ZnSc (Zinc Selenide) and subsequently data were collected in the wave number region between 4000 and 450 cm^{-1} (X axis range). The reading was further used for data analysis using Origin 9 software.

3.5. Mineral analysis

Inductively Coupled plasma Mass Spectrometry (ICPMS) of Perkin Elmer NEX ION 300X, USA had been applied for multi-elemental analysis of the sample. The samples were initially dried in a hot air oven which was digested by open air method and stored in a narrow mouth bottle and was used later for analysis.

3.5.1. Sample Digestion

The di-acid digestion procedure was followed for the digestion of sample. The peel and pomace dry powder each weighing about 0.5 g was taken separately with di acid mixture of approximately 10-15 ml prepared by mixing nitric acid and perchloric acid in the ratio of 9:4. The sample was placed in open air digestion on the hot plate till the clear white fumes were appeared and get reduced to 2-3 ml. While heating, solution was not allowed to get dried completely. The digested samples were cooled, filtered through what man No 44-filter paper and made up to the volume of 50 ml with double distilled water (DDW) in a volumetric flask and then transferred to narrow mouth bottle with details of the sample for further analysis.

3.5.2.2. Nutr

Inductively Coupled Plasma Mass Spectrometry (ICPMS) in Perkin Elmer, Nex ION 300 X) had been used for the analysis of the nutrient in the fruit peel and pomace by using system with cross flow nebulizer. The digested sample was analysed for the ionic constitution using multi elemental standard solutions no. 1, 3 and 5 supplied by Perkin Elmer containing Ag, Al, A, B, Ba , Ca, Cd, U, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Zn and Hg analytes. Further, the instrument was calibrated using standard reference material (Peach level- NIST, 1547) then the digested samples were analysed for the ionomic profiling.

3.6. Statistical analysis

All the experiment was done in triplicate. There were three citrus germplasm taken for the research work which was considered as treatment and standard statistical procedure and analytical software was applied throughout the experiment. All the experiment was performed using three factor completely randomized design with five different altitude and two stages. ANOVA was done to know about the application of the various treatment.

Chapter 4

Results

The present research entitled "Essential oil, pectin and ionome profiling of Citrus fruit waste was undertaken to evaluate three germplasms of Sikkim Himalaya. The data of the observation were subjected to three-way interaction between altitude X germplasm X fruit stage. Further two-way interaction analysis between altitude X germplasms, altitude X stages and germplasm X stages were also performed.

The experimental results are described in the following sub heads:

4.1. Collection of the samples

Three germplasms *viz:* Sikkim Mandarin (*Citrus reticulata* Blanco), Pommelo (*Citrus maxima* Merr.) and Rough Lemon (Citrus *jambhiri* Lush) were selected for the research purpose.

The fruit samples of selected germplasms in this research study were gathered from different parts of Sikkim in five different altitudes in two maturity stages (mature green and ripened). The GPS data was was taken for the study which is illustrated in Table 4.1.1.

Sl. No.	District	GPU	Longitude (E)	Latitude (N)	Altitude range (m from MSL)
1	North	Hee Gyathang	88°30’39”	27°26’44”	1420.83
		Lingdong Berfok	88°30’29”	27°29’41”	1288.33
		Lungor Sangtok	88°.29’32”	27°23’04”	1383
2	East	Bering	88°.39’34”	27°13’05”	1246
		Ranipool	88°.59’14”	27°28’51”	900
		Rorathang	88°61’15”	27°19’54 “	970
		Tadong	88°.36’13”	27°18’53”	1093
		Ranka	88° 36’18”	27 °20’36”	1650
		Assamlingzey	88°65’97”	27°26’94”	1432
3	South	Shumbuk	88°35’43”	27°19’30”	1058
		Lower Kamrang	88°33’63”	27°18’50”	800
		Upper Kamrang	88°20’96”	27°10’42”	1456
4	West	Hee Bermiok	88°19’55”	27º25’40”	1620
		Gyalshing Omchung	88°26’10”	27º27’68”	1063
		Rinchenpong	88°27’09”	27º24’33”	1700

Table 4.1.1 GPS coordinate of the places of collection of different citrus germplasms

4.2 Proximate content of peel and pomace

The proximate content of peel and pomace of three germplasms *viz:* Sikkim mandarin, Pommelo and rough lemon was analyzed. The result was illustrated in respective table along with mean, CD, SE (d) and SE (m) value.

4.2.1. Fruit weight (g)

The variation in fruit weight of three citrus germplasms collected at two stages of maturity from different altitude is shown in Table 4.2.1. The highest fruit weight was observed in pommelo (G_2) followed by Sikkim mandarin (G_1) and rough lemon (G_3). There was increasing trend in fruit weight with increased altitude range in Sikkim mandarin. However, no such trend was observed in pommelo and rough lemon. The fruit weight was higher for ripened fruit than the mature green stage, irrespective of germplasms and altitude. Fruit weight in Sikkim mandarin was highest

(88.56 g) at 1200-1400 m altitude and lowest i.e. 49.78 g at 800-1000 m altitude. The highest fruit weight in Pommelo and rough lemon was recorded up to 1,344.00 g and 57.67 g, respectively at 1400-1600 m altitude collected at ripened stage.

4.2.2. Diameter (mm)

The diameter of three citrus germplasm is shown in Table 4.2.1, wherein it is depicted that the maximum diameter was recorded for pommelo (242.70 mm) at ripened stage. Whereas the Sikkim mandarin and rough lemon were found to be 52.33 mm (1200-1400 m altitude) and 107 mm (1400-1600 m altitude) at ripened stage, respectively. On the other hand, the lowest diameter was 44.89 mm at 800-1000 m altitude at mature green stage of Sikkim mandarin. There was increase in diameter up to 1200-1400 m altitude and showed a decrease pattern at 1400-1600 m altitude which eventually reaches to maximum value at >1600 m in all the germplasms except for pommelo and rough lemon in mature green stage. The fruit in ripened stage showed higher diameter than the mature green stage.

4.2.3. Number of segments

The data regarding number of segments varied from 8.33 in ripened stage of rough lemon to 22 number of segments in Pommelo (1400-1600 m altitude) had highest of 22 number of segments. Further increase in number of segments was observed only for pommelo at ripened stage. However, Sikkim mandarin, rough lemon and pommelo (mature green stage) were found with different trend with increase in altitude. In addition, the lowest content in number of segments was found from rough lemon at mature green stage in 1400-1600 m altitude (Table 4.2.1).

4.2.4. Vitamin C (mg/100g):

The data regarding vitamin C is depicted in Table No. 4.2.1. There was increasing trend of vitamin C with increasing altitude for Sikkim mandarin. However, no such pattern was noticed for pommelo and rough lemon. Further vitamin C content was much higher for mature green fruit than the ripened one irrespective of germplasm and altitude.

The vitamin C was highest in rough lemon followed by pommelo and Sikkim mandarin. In Rough lemon, highest vitamin C (118.33 mg/100g) was recorded in the fruits collected from 1200-1400 m altitude, whereas in Sikkim mandarin highest vitamin C was 37.37 mg/100g in the fruits collected at 800-1000 m altitude.

Table 4.2.1 Variation in fruit parameters of three different germplasms of citrus at different maturity stages grown at different altitude

Germplasm	Altitude (m)	Fruit weight (g)		Diameter(mm)		No of Segment		Vitamin C (mg/100g)	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	63.00	49.78	44.89	49.00	9.40	9.43	47.00	37.37
	1000-1200	60.00	67.79	51.45	49.67	9.73	9.67	48.80	38.80
	1200-1400	86.33	88.56	51.43	52.33	10.47	10.40	52.00	39.26
	1400-1600	69.67	85.33	50.00	51.67	9.40	9.66	52.90	40.16
	>1600	71.00	84.22	51.44	54.67	10.80	10.83	53.02	40.40
Pommelo	800-1000	1,025.00	1,145.00	111.67	137.33	17.00	17.00	81.44	62.00
	1000-1200	881.67	903.33	128.33	128.70	16.66	17.66	62.67	59.42
	1200-1400	935.00	983.33	156.00	241.67	18.33	20.00	79.38	72.14
	1400-1600	921.67	1,344.00	215.00	203.67	18.00	21.66	75.04	72.67
	>1600	1,222.67	945.00	237.89	242.70	19.66	22.00	91.58	81.33
Rough lemon	800-1000	41.00	43.00	70.67	76.33	9.33	8.33	92.22	90.33
	1000-1200	47.00	52.00	66.67	95.33	9.33	9.00	113.00	108.00
	1200-1400	52.67	57.37	78.52	95.00	10.33	10.33	118.33	114.67
	1400-1600	53.00	57.67	86.67	107.00	8.00	10.67	82.00	81.00
	>1600	43.00	47.00	71.67	83.32	9.67	8.66	110.50	105.00

Factors	C.D (5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)
Factor (G)	1.60	0.80	0.56	1.71	0.85	0.60	0.42	0.21	0.15	0.99	0.50	0.35

Factor (A)	**2.07**	**1.03**	**0.73**	**2.20**	**1.10**	**0.78**	**0.54**	**0.27**	**0.19**	**1.28**	**0.64**	**0.45**
Factor (S)	**1.31**	**0.65**	**0.46**	**1.39**	**0.70**	**0.49**	**0.34**	**0.16**	**0.12**	**0.81**	**0.40**	**0.29**
Interaction GXAX S	**5.06**	**2.53**	**1.79**	**5.39**	**2.70**	**1.91**	**1.33**	**0.65**	**0.46**	**3.13**	**1.57**	**1.11**

4.2.5. Total Soluble Solids (° B)

The TSS content of all the fruit germplasm was found higher at the ripened stage than the mature green stage and amongst the germplasms, pommelo was recorded with highest TSS followed by Sikkim mandarin and rough lemon. The TSS content up to 13.66°B was estimated for Sikkim mandarin at >1600 m altitude. In pommelo11.66°B was recorded as highest TSS at 1200-1400 m altitude. Likewise, 8.33° B was recorded as highest TSS at >1600 m altitude for rough lemon. However, amongst all the three germplasm, Sikkim mandarin (13.66°B) hold the maximum value in ripened stage at >1600 m altitude. Moreover, the trend for TSS content with respect to altitude was similar for all the germplasm except for Sikkim mandarin as it showed increase in TSS with increasing altitude. As compared to mature green stage, ripened stage showed higher TSS in all the germplasm (Table 4.2.2)

4.2.6. Titrable Acidity (TA)

In an analysis of variance for three factors, TA content was found to be significantly high in rough lemon at 1200-1400 m altitude (2.33) compared to all other genotypes. Furthermore, pommelo (2.23) at 1000-1200 m altitude was found to be at par with rough lemon. On the contrary, Sikkim mandarin (1.65) at 1200-1400 m altitude at mature green showed the lowest titrable acidity. In addition, there is no increasing trend with increase in altitude for all the germplasm as illustrated in (Table 4.2.2). The interaction of germplasm and stages showed mature green stage being with higher titrable acidity than ripened stage.

In all the germplasm, ripened stage was found to be considerably higher except in case of rough lemon which showed the different trend.

4.2.7. TSS: Acid

TSS: acid ratio was recorded highest in Sikkim mandarin fruits (11.88) collected from >1600 m altitude at ripened stage. The ratio was found highest in Sikkim mandarin than other germplasms. In pommelo highest ratio (9.16) was recorded in the fruit collected from 1400-1600 m altitude. The rough lemon had the lowest TSS: acid (6.23) at >1600 m altitude in ripened stage, though the values was lesser than values recorded for Pommelo and Sikkim mandarin collected from all the altitude range. The TSS: acid was higher during the ripening stage.

Comparison of germplasm with stage showed, Sikkim mandarin and pommelo holding higher TSS: acid in ripened stage compared to mature green stage. However, rough lemon was different. The germplasm did not show any trend with increasing altitude. It was also found that >1600 m altitude in ripened stage showed the maximum TSS: acid (Table 4.2.2)

Table 4.2.2: Chemical parameters of three different citrus germplasms at different maturity stages grown at different altitude

Germplasm	Altitude (m)	TSS (°B)		Titrable acidity		TSS: Acid	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	10.83	12.00	1.30	1.17	8.33	10.26
	1000-1200	10.33	11.33	1.57	1.17	6.58	10.54
	1200-1400	11.66	11.66	1.65	1.20	7.07	9.72
	1400-1600	11.00	12.33	1.46	1.11	7.53	10.21
	>1600	12.33	13.66	1.50	1.15	8.22	11.88
Pommelo	**800-1000**	10.50	10.76	2.09	1.77	5.02	6.08
	1000-1200	10.80	11.33	2.23	1.57	4.84	7.22
	1200-1400	10.44	11.66	2.07	1.37	5.04	8.51
	1400-1600	10.02	10.17	1.83	1.11	5.48	9.16
	>1600	10.33	11.33	1.79	1.37	5.77	8.27
Rough lemon	**800-1000**	6.43	6.53	1.65	1.73	3.90	3.77
	1000-1200	7.30	7.30	1.66	1.76	4.40	4.15
	1200-1400	7.43	8.30	2.33	2.27	3.19	3.66
	1400-1600	7.66	6.00	2.30	2.21	2.71	3.33
	>1600	7.60	8.33	1.60	1.67	4.75	4.99

Factors	C.D (5%)	SE(d)	SE(m)	C.D (5%)	SE (d)	SE(m)	C.D (5%)	SE(d)	SE(m)
Factor(G)	0.27	0.14	0.10	0.03	0.02	0.01	0.24	0.12	0.09
Factor(A)	0.35	0.18	0.13	0.04	0.02	0.02	0.31	0.15	0.11
Factor(S)	0.22	0.11	0.08	0.03	0.01	0.01	0.20	0.10	0.07
Interaction (G X A X S)	0.86	0.43	0.31	0.10	0.05	0.04	0.76	0.38	0.27

4.2.8. Reducing Sugar (%)

The three-way interaction (Table 4.2.3) revealed that the fruit of Sikkim mandarin collected from >1600 m altitude at ripened stage had the maximum reducing sugar (7.00 %) content than the fruits collected from other altitude. The reducing sugar was recorded highest in Sikkim mandarin than other germplasms. Pommelo collected from >1600 m altitude had reducing sugar during ripened stage was to the tune of 6.31 %, the value was higher than that of other altitudes.

The lowest record of reducing sugar was 5.06 % in the pommelo collected from 800-1000 m altitude in mature green stage. Lowest reducing sugar amongst the germplasms was recorded in rough lemon. Higher altitude (>1600 m) had higher reducing sugar (2.98 %) at ripened stage. The reducing sugar was as low as 2.37 % at 1000-1200 m altitude in mature green stage fruit.

It was also revealed that all the germplasm showed increasing pattern with increase in altitude and the highest value being recorded for the fruits collected from >1600 m altitude. In all the experiment, ripened stage of fruit had higher reducing sugar than the mature green stage.

4.2.9. Total sugar (%)

The variation in the total sugar content of three germplasms of citrus at different altitudinal range at two different stages *viz:* ripened and mature green stages are shown in Table 4.2.3. The highest total sugar was recorded in pommelo followed by Sikkim mandarin and rough lemon. All the germplasms had highest total sugar at the altitudinal range >1600 m *viz.* Sikkim mandarin (9.20 %), pommelo (8.69 %) and rough lemon (4.63 %). Increasing trend of total sugar with increased altitude was observed in Sikkim mandarin and pommelo. However, no such pattern was seen in rough lemon. It was also found that total sugar was much higher in ripened fruit than the mature green irrespective of germplasm and altitude. In Sikkim mandarin and pommelo, total sugar was highest at an altitude of >1600 m. Amongst all the factors, lowest total sugar content was recorded for rough lemon (2.51 %) collected from 1000-1200 m altitude in mature green stage.

4.2.10. Non reducing sugar (%)

The ripened stage of the fruit was recorded with higher non reducing sugar content in than the mature green stage in Sikkim mandarin and pommelo. Though the trend in rough lemon was different. The samples collected from the higher altitude (> 1600 m) had the higher non- reducing sugar than other altitudinal range in all the germplasms *viz.* Pommelo (2.26 %) and rough lemon (1.57 %) and Sikkim mandarin (2.09 %) at ripened stage as revealed from the three-way interaction (Table No 4.2.3).

Table 4.2.3: Comparision of chemical parameters amongs different citrus germplasm grown at different maturity stages and at different altitudes

Germplasm	Altitude (m)	Reducing Sugar (%)		Total Sugar (%)			Non-Reducing sugar (%)		
		Mature green	Ripened	Mature green		Ripened	Mature green		Ripened
Sikkim mandarin	**800-1000**	5.08	5.89	7.07		8.03	1.89		2.03
	1000-1200	6.18	6.39	7.29		8.28	1.05		1.80
	1200-1400	6.05	6.63	7.81		8.80	1.67		2.06
	1400-1600	6.07	6.67	7.87		8.10	1.71		1.36
	>1600	6.31	7.00	8.27		9.20	1.86		2.09
Pommelo	**800-1000**	5.06	5.21	6.30		7.04	1.18		1.74
	1000-1200	5.37	5.48	6.25		7.12	0.84		1.56
	1200-1400	5.52	5.62	7.03		7.27	1.43		1.57
	1400-1600	5.74	6.00	7.32		7.98	1.50		1.88
	>1600	6.17	6.31	7.91		8.69	1.65		2.26
Rough lemon	**800-1000**	2.40	2.50	2.80		3.17	0.38		0.64
	1000-1200	2.37	2.40	2.51		2.58	0.13		0.17
	1200-1400	2.53	2.60	3.08		3.15	0.52		0.52
	1400-1600	2.70	2.81	3.46		3.53	0.72		0.68
	>1600	2.90	2.98	4.30		4.63	1.33		1.57
Factors	**C.D (5%)**	**SE(d)**	**SE(m)**	**C.D (5%)**	**SE(d)**	**SE(m)**	**C.D (5%)**	**SE(d)**	**SE (m)**
Factor(G)	**0.08**	**0.04**	**0.03**	**0.06**	**0.03**	**0.02**	**0.08**	**0.04**	**0.03**
Factor(A)	**0.10**	**0.05**	**0.04**	**0.07**	**0.04**	**0.03**	**0.10**	**0.05**	**0.04**
Factor(S)	**0.06**	**0.03**	**0.02**	**0.05**	**0.02**	**0.02**	**0.07**	**0.03**	**0.02**
Interaction (G X A X S)	**0.25**	**0.13**	**0.09**	**0.17**	**0.09**	**0.06**	**0.26**	**0.13**	**0.09**

4.2.11. Peel moisture (%)

Three factor analysis of variance is shown in Table No. 4.2.4 which showed Sikkim mandarin being superior having highest peel moisture up to (68.77 %) at an altitude range of 1400-1600 m at ripened stage followed by 66.46 % at 1000- 1200 m altitude.

In Pommelo, peel moisture was recorded up to 50 %, whereas in rough lemon it was 44 % at altitude range >1600 m. The lowest peel moisture content during the experiment was observed in pommelo (32.67 %) at 1000-1200 m altitude in mature green stage.

The mature green stage of Sikkim mandarin showed increasing peel moisture content with increase in altitude. Whereas the rest of germplasms didnot show uniform pattern. However, the highest peel moisture in all the germplasm was observed at highest altitude i.e. >1600 m. In all the cases, ripened stage of the fruit was recorded with higher peel moisture than the mature green stage.

4.2.12. Pomace moisture (%)

The pomace moisture as revealed from the three-way interaction of germplasm X altitude X stage of maturity (Table No. 4.2.4). Pomace of rough lemon was observed to have 47.67 % at 800-1000 m altitude at ripened stage whereas the lowest was recorded for mature green Pommelo. (28.33 %) collected from 1000-1200 m altitudes.

Increasing altitude had increased pomace moisture in Sikkim mandarin and pommelo, whereas rough lemon was not showing such trend at mature green as well as ripened stage.

4.2.13. Peel Crude fat (%)

The interaction between altitude, germplasm and fruit stages was illustrated in Table No 4.2.4. It showed highest crude fat content in the ripened peel of Sikkim mandarin collected at >1600 m altitude with an estimate of 19.07 % followed by pommelo (16.29 %) and rough lemon (13.20 %) at >1600 m altitude It is interesting to note that the peel crude fat was highest at the higher altitude.

The comparison between stages for germplasm revealed ripened stage with higher crude fat peel than mature green stage.

4.2.14. Pomace crude fat (%)

The factor analysis of variance (Table No. 4.2.4) revealed that the pomace crude fat was highest (19.33 %) in rough lemon collected from 1000-1200 m altitude at ripened stage. Significantly lower crude fat was estimated in the sample of Sikkim mandarin (15.30 %) and pommelo (15.00 %) at altitude >1600 m. On the other hand, pomace crude fat was as low as 10 % in mature green samples at 1200-1400 m in pommelo.

On comparison amongst stages of fruit maturity, pomace of ripened fruit had higher crude fat than the mature green stage for all the studied germplasms.

Table No 4.2.4: Characteristics of peel and pomace of citrus germplasms at different maturity stages grown at different altitude

Germplasm	Altitude (m)	Peel moisture (%)		Pomace moisture (%)		Crude peel fat(%)		Crude pomace fat (%)	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	42.86	48.12	34.53	30.67	15.48	12.33	13.63	12.37
	1000-1200	66.46	48.23	34.53	31.00	18.42	17.60	15.30	14.72
	1200-1400	50.84	49.32	37.48	34.41	17.98	12.63	16.40	14.81
	1400-1600	68.77	59.90	43.22	35.52	18.63	18.01	12.83	13.00
	>1600	69.00	60.17	43.93	35.52	19.07	11.39	12.70	12.17
Pommelo	**800-1000**	42.33	37.33	36.00	34.43	14.80	14.33	13.78	10.33
	1000-1200	41.28	32.67	37.67	28.33	16.67	12.87	10.50	10.07
	1200-1400	48.15	36.64	33.00	29.67	18.00	13.66	10.77	10.00
	1400-1600	44.33	37.26	36.33	29.67	16.35	14.93	13.72	11.33
	>1600	50.00	38.03	36.38	33.67	17.33	16.29	15.00	13.00
Rough lemon	**800-1000**	42.87	39.33	47.67	43.00	13.72	13.23	19.33	16.33
	1000-1200	40.79	38.00	40.67	40.00	13.58	13.20	16.73	14.66
	1200-1400	40.00	37.33	44.72	42.33	13.20	12.62	17.23	15.00
	1400-1600	40.99	37.33	44.88	41.67	13.40	12.17	15.67	14.00
	>1600	44.00	41.67	41.17	40.00	13.20	12.62	15.33	12.66

Factors	C.D (5%)	SE(d)	SE (m)	C.D(5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE (m)
Factor(G)	**0.78**	**0.39**	**0.27**	**0.79**	**0.39**	**0.28**	**0.28**	**0.14**	**0.10**	**0.32**	**0.16**	**0.11**
Factor(A)	**1.00**	**0.50**	**0.35**	**1.03**	**0.51**	**0.36**	**0.36**	**0.18**	**0.13**	**0.41**	**0.20**	**0.14**
Factor(S)	**0.63**	**0.32**	**0.22**	**0.65**	**0.32**	**0.23**	**0.23**	**0.11**	**0.08**	**0.26**	**0.13**	**0.09**
Interaction (G X A X S)	**2.45**	**1.24**	**0.87**	**2.52**	**1.26**	**0.89**	**0.89**	**0.45**	**0.32**	**0.99**	**0.49**	**0.35**

4.2.15. Peel crude fibre (%)

The crude fibre of fruit peel was found to be highest (27.44 %) at the ripened stage of Sikkim mandarin grown at an altitude of >1600 m compared to other germplasms grown at different altitudes (Table 4.2.5). Further, Pommelo had the highest peel crude fibre to the tune of 25.47 % at >1600 m altitude followed by rough lemon i.e. 22.87 % collected from 1000-1200 m altitude at ripened stage. On the other hand, lowest peel crude fibre was 11.24 % at mature green Sikkim mandarin collected from 800-1000 m altitude. The peel fibre was comparatively higher for ripened fruit than that of mature green fruit irrespective of the altitude and stages.

4.2.16. Pomace Crude fibre (%)

The crude fibre of pomace was recorded highest (27.67 %) in the ripened stage Pommelo and rough lemon fruit collected from altitude 1400-1600 m. The data was at par with the recorded data from other altitude. Whereas the lowest crude fibre was recorded in the pomace of Sikkim mandarin (11.59 %) at 1000-1200 m altitude in mature green stage (Table 4.2.5).

4.2.17. Peel Ash (%)

The data regarding peel ash as shown in Table No 4.2.5 revealed that the Sikkim mandarin at ripened stage collected from >1600 m altitude had the highest value of 27.44 % amongst all the interactions. Whereas the least value was recorded for rough lemon collected at mature green stage from 1000-1200 m altitude (9.57 %). Pommelo peel was recorded with 24.33 % ash at 1400-1600 m altitude and 16.33 % at 800-1000m altitude in mature green stage as the highest and lowest content in peel ash.

There was increasing trend in peel ash content with increased altitude in case of Sikkim mandarin and pommelo. However, no such trend was found for rough lemon.

Moreover, ripened fruit shows much higher peel ash content as compared to mature green stage irrespective of the different germplasm and stages.

4.2.18. Pomace Ash (%)

The ash content in pomace was highest in rough lemon collected from > 1600 m (41.10 %) at ripened stage which is statistically at par with 40.65 % in rough lemon collected from 800-1000 m altitude and 39.67 % in pommelo at 1200-1400 m altitude in ripened stage. In mature green stage, highest pomace ash (37.67 %) was estimated in the rough lemon samples collected from > 1600 m and lowest was in the samples of Sikkim mandarin (12.73 %) collected from 1200- 1400 m from MSL (Table 4.2.5)

There was no pattern of increase in pomace ash with respect to increasing altitude in all the germplasms. Pomace ash was found to be highest for ripened fruit than the mature green stage irrespective of the germplasms and altitude.

Germplasm	Altitude (m)	Crude peel fibre (%)		Crude pomace fibre (%)		Peel ash (%)		Pomace ash (%)	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	16.03	11.24	17.87	19.07	11.51	15.61	15.08	17.40
	1000-1200	18.12	11.41	20.65	11.59	11.53	17.91	13.67	20.57
	1200-1400	20.47	15.73	20.67	11.74	15.30	17.93	12.73	18.54
	1400-1600	23.96	16.23	21.52	15.09	17.70	24.16	18.07	21.03
	>1600	27.44	21.40	21.76	15.16	20.13	27.44	13.65	20.40
Pommelo	800-1000	20.28	18.33	22.49	26.85	16.33	17.00	29.33	30.67
	1000-1200	18.73	16.95	26.89	26.86	16.67	18.14	31.67	32.30
	1200-1400	22.51	18.40	25.00	25.31	21.67	22.00	34.33	39.67
	1400-1600	19.33	18.73	20.67	20.67	23.00	23.00	29.67	32.33
	>1600	25.47	22.33	25.40	27.67	23.67	24.33	31.33	34.14
Rough lemon	800-1000	22.80	19.60	13.92	11.98	10.73	13.00	33.67	40.65
	1000-1200	22.87	18.76	17.51	15.53	9.57	10.33	32.33	36.17
	1200-1400	20.80	18.33	21.35	23.56	11.13	12.33	36.00	36.53
	1400-1600	16.93	16.93	23.25	27.67	11.73	13.00	34.66	35.67
	>1600	20.85	20.33	23.00	20.67	11.13	12.67	37.67	41.10

Factors	C.D (5%)	SE(d)	SE(m)	C.D (5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)
Factor(G)	0.54	0.27	0.19	0.79	0.39	0.28	0.60	0.29	0.21	0.63	0.31	0.22
Factor(A)	0.69	0.35	0.25	1.03	0.51	0.36	0.77	0.38	0.27	0.81	0.40	0.29
Factor(S)	0.44	0.22	0.16	0.65	0.32	0.23	0.49	0.24	0.17	0.51	0.26	0.18
Interaction (G X	1.70	0.85	0.60	2.52	1.26	0.89	1.89	0.93	0.66	1.98	0.99	0.70

A X S)												

Table No 4.2.5: Characteristics of peel and pomace of citrus germplasms at different maturity stages grown at five different altitudes

4.2.19. Peel Starch (%)

Pommelo fruit samples collected from > 1600 m altitude was recorded with highest peel starch (6.37 %) at mature green stage, and the value is highest amongst all other germplasms collected from different altitude as compared to rest of the germplasm (Table 4.2.6). There was no significant difference in the starch content estimated for ripened stage at same altitude (6.35 %). Highest starch content in Sikkim mandarin was recorded in the samples collected from > 1600 m altitude at mature green stage. In rough lemon, the highest value was seen to be 2.52 % in the mature green samples collected from 1200 to 1400 m altitude. The lowest record was however, 1.23 %, 3.85 % and 1.11 %, respectively for ripened fruit samples of Sikkim mandarin, Pommelo and Rough lemon.

4.2.20. Pomace Starch (%)

The highest starch in pomace was 6.22 % in mature green pommelo fruits collected from 1400-1600 m which was followed by 5.74 % in mature green stage pommelo collected from 1000-1200 m. The lowest content was also recorded in Sikkim mandarin (1.06 %) of ripened stage collected from 800-1000 m altitude (Table 4.2.6).

The increase in pomace starch was irrespective of increase in altitude in all the germplasms. Further, pomace starch was much higher for mature green fruit than ripened stage for all the germplasms.

4.2.21. Peel Protein (%)

The highest protein content (3.12 %) in peel was in Pommelo in its mature green stage at altitude 800-1000 m but not significantly higher than that of Sikkim mandarin in its ripened stage at 800-1000 m (3.11 %) and at >1600 m (3.05 %). In rough lemon samples, peel protein was 2.75 % at 1200-1400 m, a highest value at ripened stage and 1.93 % (800-1000 m) as the lowest in mature green stage. There is no trend for increase in peel protein with respect to increase in altitude. Moreover, ripened stage showed much higher peel protein content than mature green stage (Table 4.2.6).

4.2.22. Pomace Protein (%)

The highest pomace protein was recorded in pommelo followed by Sikkim mandarin and rough lemon. There was increasing trend with altitude for rough lemon both at ripened and mature green stages. However, no such trend was observed for Sikkim mandarin and pommelo (Table 4.2.6). The pomace protein was also found to be much higher for ripened fruit than the mature green stage irrespective of germplasm and altitude. In Sikkim mandarin pomace protein was highest i.e. 3.00 % at 1400-1600 m altitude at ripened stage. Likewise, pommelo and rough lemon with 3.06 % and 2.83 % at >1600 m altitude as the highest. However, pommelo showed the maximum amongst all the germplasm.

Table No 4.2.6: Starch and protein content in peel and pomace of citrus germplasm at different maturity stages grown at five different altitude

Germplasm	Altitude (m)	Peel starch (%)		Pomace starch (%)		Peel protein (%)		Pomace protein (%)	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	1.83	1.23	1.06	1.95	2.65	3.11	2.10	2.34
	1000-1200	2.31	2.52	1.17	1.61	2.10	2.94	2.25	2.27
	1200-1400	2.33	3.03	1.37	1.80	2.23	2.77	2.30	2.41
	1400-1600	2.63	3.05	1.33	1.76	2.26	2.63	2.11	3.00
	>1600	3.40	3.17	1.20	1.82	2.24	3.05	2.19	2.39
Pommelo	**800-1000**	4.10	4.41	4.59	5.40	2.31	3.12	1.89	2.32
	1000-1200	5.67	5.57	5.74	4.77	2.25	2.62	2.70	2.75
	1200-1400	5.32	4.03	4.49	4.05	2.33	2.48	2.48	3.03
	1400-1600	4.11	3.85	6.22	4.73	1.50	1.62	2.34	2.83
	>1600	6.37	6.35	4.54	5.46	2.02	2.48	2.47	3.06
Rough lemon	**800-1000**	1.48	1.41	1.93	1.32	1.93	2.13	1.90	2.11
	1000-1200	1.57	2.16	2.57	1.36	2.10	2.21	2.10	2.30
	1200-1400	2.32	1.11	1.34	1.29	2.23	2.75	2.36	2.60
	1400-1600	2.13	1.38	1.69	1.57	2.39	2.46	2.51	2.78
	>1600	1.73	1.66	2.75	2.62	2.37	2.43	2.69	2.83

Factors	**C.D (5%)**	**SE(d)**	**SE(m)**	**C.D(5%)**	**SE(d)**	**SE(m)**	**C.D (5%)**	**SE(d)**	**SE(m)**	**C.D (5%)**	**SE(d)**	**SE(m)**
Factor(G)	**0.08**	**0.04**	**0.03**	**0.04**	**0.02**	**0.02**	**0.08**	**0.04**	**0.03**	**0.05**	**0.03**	**0.02**
Factor(A)	**0.10**	**0.05**	**0.04**	**0.06**	**0.03**	**0.02**	**0.10**	**0.05**	**0.04**	**0.07**	**0.03**	**0.02**
Factor(S)	**0.06**	**0.03**	**0.02**	**0.04**	**0.02**	**0.01**	**0.06**	**0.03**	**0.02**	**0.04**	**0.02**	**0.02**
Interaction (G X A X S)	**0.25**	**0.12**	**0.09**	**0.14**	**0.07**	**0.05**	**0.24**	**0.12**	**0.09**	**0.16**	**0.08**	**0.06**

4.3. Antioxidants

4.3.1. Peel total Phenol (mg/GAE/g)

The three way interactions of germplasms, altitude range and the stage of maturity (Table 4.2.7), depicts the presence of highest total phenol (244.90 mg/GAE/g)) in ripened stage of Sikkim mandarin grown at >1600 m as compared to all other interactions, though there was at par value of the total phenol in the peel of ripened Sikkim mandarin grown at 1200-1400 m (244.60 mg/GAE/g) and 1400-1600 m (241.68 mg/GAE/g) . On the other hand, highest total phenol was found with (124.02 mg/GAE/g) at >1600 m altitude in pommelo and 121.01 mg/GAE/g at 1400-1600 m attitude in rough lemon at ripened stage. There was increasing trend for pommelo with increasing altitude. However, no such pattern was found in Sikkim mandarin and rough lemon. In all the cases, ripened fruit peel had higher total phenol the mature green stage irrespective of germplasms and altitude.

4.3.2. Pomace total phenol (mg/GAE/g)

The pomace of mature green pommelo grown at >1600 m altitude was recorded with highest total phenol to the tune of 125.07 mg/GAE/g). Whereas, the pomace of ripened pommelo fruit grown at > 1600 m altitude recorded total phenol 122.86 mg/GAE/g. In addition, Sikkim mandarin showed highest total phenol (121.29 mg/GAE/g) and rough lemon it was 124.66 mg/GAE/g at 1400-1600 m altitude. In both the cases, pomace from ripen fruits had higher value than the mature green stage (Table 4.2.7)

4.3.3. Peel antioxidant activity by DPPH method (%)

The highest antioxidant activity (DPPH method) was recorded in peel of ripened Sikkim mandarin followed by pommelo and rough lemon. Sikkim mandarin grown at 1400-1600 m was recorded with 93.33 % DPPH and at par value (92.33 %)

in the sample collected at mature green stage from > 1600 m altitude as shown in Table 4.2.7.

Furthermore, pommelo was recorded with 92.67 % (>1600 m) altitude in ripened stage being next highest after Sikkim mandarin, at par values were recorded to be 92.29 % (800-1000 m in pommelo), 92.67 % (>1600m in pommelo) and 92.33 % (1200-1400 m in pommelo) in mature green stage. In rough lemon, highest antioxidant activity (DPPH method) was 91.35 % (collected from >1600 m altitude) at ripened stage.

The increase in peel antioxidant activity (DPPH method) was not in uniformity with increasing altitude. Likewise, peel antioxidant activity (DPPH method) was also found much higher for ripened fruit than the mature green stage irrespective of altitude and germplasm.

4.3.4. Pomace antioxidant activity by DPPH method (%)

The pomace of ripened samples of Sikkim mandarin collected from 1200-1400 m altitude accounted for highest antioxidant activity (DPPH method) (95.47 %) which was found to be at par with (95.03 %) at >1600 m altitude. It was also found to be at par with rough lemon with 93.96 % (800-1000 m), 94.73 % (1000-1200 m) and 92.67 % (>1600 m altitude) in mature green stages. On the other hand, highest antioxidant activity (DPPH method) in pommelo was 80.74 % (>1600 m altitude) and rough lemon with 94.73 % (1000-1200 m altitude). Whereas the lowest was 70.67 % (800-1000 m altitude) in mature green stage for pommelo out of all the 30 combination (Table 4.2.7)

There was no such trend of increase in pomace antioxidant activity (DPPH method) with increase in altitude for all the germplasm. The ripened stage also showed much higher pomace antioxidant activity (DPPH method) as compared to mature green stage irrespective of all the germplasm and two stages.

Table No 4.2.7: Total phenol and antioxidant properties of peel and pomace of citrus germplasm at different maturity stages grown at different altitude

Germplasm	Altitude (m)	Peel Phenol (mg/g)		Pomace phenol (mg/g)		Peel Antioxidant (DPPH method) %		Pomace Antioxidant (DPPH method) %	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	228.80	206.74	107.00	104.45	86.00	81.16	84.86	84.67
	1000-1200	237.31	214.67	108.57	105.42	87.67	79.93	87.47	85.00
	1200-1400	244.60	218.33	111.21	107.85	89.49	71.02	95.47	88.33
	1400-1600	241.68	217.37	113.49	112.06	93.33	71.92	82.67	81.39
	>1600	244.90	220.25	121.29	113.56	92.33	73.62	95.03	90.33
Pommelo	**800-1000**	113.31	107.59	115.70	107.72	92.29	73.95	72.67	70.67
	1000-1200	118.91	117.65	118.80	117.13	89.67	84.13	80.00	74.33
	1200-1400	121.36	120.98	121.00	117.56	89.32	92.33	77.88	73.67
	1400-1600	123.69	121.00	123.94	120.00	89.76	85.25	73.00	73.33
	>1600	124.02	121.89	125.07	122.86	92.67	78.99	80.74	77.88
Rough lemon	**800-1000**	114.91	114.74	110.84	109.79	88.72	74.39	93.96	83.00
	1000-1200	119.23	116.87	122.42	113.21	87.70	72.33	94.73	90.33
	1200-1400	117.33	119.42	124.47	116.40	87.29	80.61	75.69	74.49
	1400-1600	121.01	119.99	124.66	122.37	82.68	70.81	87.83	79.67
	>1600	116.04	111.65	120.02	113.12	91.35	70.56	92.67	91.99

Factors	**C.D (5%)**	**SE(d)**	**SE(m)**	**C.D(5%)**	**SE(d)**	**SE(m)**	**C.D (5%)**	**SE(d)**	**SE(m)**	**C.D(5%)**	**SE(d)**	**SE(m)**
Factor(G)	**1.62**	**0.81**	**0.57**	**1.14**	**0.57**	**0.40**	**0.99**	**0.49**	**0.35**	**0.86**	**0.43**	**0.30**
Factor(A)	**2.09**	**1.05**	**0.74**	**1.47**	**0.74**	**0.52**	**1.28**	**0.64**	**0.45**	**1.10**	**0.56**	**0.39**
Factor(S)	**1.32**	**0.66**	**0.47**	**0.93**	**0.47**	**0.33**	**0.81**	**0.40**	**0.28**	**0.70**	**0.35**	**0.25**
Interaction (G X A X S)	**4.87**	**2.56**	**1.81**	**3.60**	**1.79**	**1.27**	**3.13**	**1.57**	**1.11**	**2.72**	**1.36**	**0.96**

4.3.5. Peel IC_{50} (mg/ml)

Out of the 30 combination in 3-way interaction 0.81mg/ml in pommelo at >1600 m altitude (mature green stage) and 2.78 mg/ml in rough lemon at 1200-1400 m altitude (ripened stage) was recorded with the lowest and highest peel IC_{50}. However, in ripened stage, Sikkim mandarin was recorded with 1.97 mg/ml (1200-1400 m altitude) as the highest IC_{50} for peel. There was uniform increasing trend of peel IC_{50} with increase in altitude for pommelo. However, no such trend was observed for Sikkim mandarin and rough lemon. Furthermore, ripened stage showed much higher peel IC_{50} than mature green stage irrespective of germplasm and altitude.

It was also noted that germplasm 3 (rough lemon) in ripened stage at 1200-1400 m altitude showed the highest peel IC_{50} and that of least being occupied by germplasm 2 (0.81%) at mature green stage at 800-1000 m altitude (Table 4.2.8).

4.3.6. Pomace IC_{50} (mg/ml)

The higher pomace IC_{50} was observed in Sikkim mandarin followed by pommelo and rough lemon (Table 4.2.8). The highest IC_{50} was recorded in the ripened samples collected from >1600 m (3.83 mg/ml) followed by Sikkim mandarin samples at 1200-1400 m (3.59 mg/ml). The lowest record was 0.68 mg/ml for the rough lemon samples at ripened stage collected from >1600 m altitude) and the highest was 1.70 mg/ml in ripened stage at 1400-1600 m.

In pommelo highest value was 2.14 mg/ml at 800-1000 m altitude in ripened stage. In regard to altitude, there was trend of increase with increasing altitude. Moreover, peel IC_{50} was found to be higher in ripened stage than mature green irrespective of germplasms and altitude.

4.3.7. Peel flavonoid (mg/QE/g)

The maximum peel flavonoid (187.34mg/QE/g) was recorded in rough lemon samples collected at 1200-1400 m altitude in ripened stage. Similarly, in mature green stage maximum value was 179.85 mg/ml collected from 1400-1600 m altitude (Table 4.2.8)

Likewise, pommelo was recorded with 180 mg/QE/g and 178.67 mg/QE/g at 1000-1200 m altitude as the highest value at ripened and mature green stage. Moreover, Sikkim mandarin was also found with 156 mg/QE/g (>1600 m altitude) and 146.79 mg/QE/g (1400-1600 m altitude) as the highest for ripened and mature green stages.

There was also uniform pattern of increase in peel flavonoid with respect to increase in altitude. However, no such trend was found for pommelo and rough lemon. The peel flavonoid was also found to be higher for ripened fruit than that of mature green stage fruit.

Out of all the 30 combination, 187.34 mg/QE/g in rough lemon at 1200-1400 m altitude in ripened stage was the highest recorded value. Whereas the least was found in rough lemon at >1600 m altitude (111.43 mg/QE/g).

4.3.8. Pomace flavonoid (mg/QE/g)

The flavonoid in the pomace of pommelo fruit was as high as 201.67 mg/QE/g in the ripened samples collected from 1400-1600 m altitude closely followed by 194 mg/QE/g at >1600 m altitude in ripened stage and that of the lowest value was recorded in Sikkim mandarin (157 mg/QE/g) at mature green stage as shown in three-way combination (Table No 4.2.8). In regard to Sikkim mandarin, flavonoid content was as high as 168.33 mg/QE/g in the ripened samples collected from 1200-1400 m altitude. The lowest record was 154.33 mg/QE/g (>1600 m) altitude in mature green stage. Likewise, rough lemon was observed with maximum value of 174.97 mg/QE/g at >1600 m altitude at ripened stage and the minimum values of 159.67 mg/QE/g at 1400-1600 m altitude at mature green stage.

In pommelo, the maximum flavonoid content was found in the pomace of ripened fruit samples collected from 1400-1600 m altitude 201.67 mg/QE/g) and the minimum of flavonoid content was recorded in the mature green stage samples (154.33 mg/QE).

Table No 4.2.8: IC_{50} and flavonoid content in peel and pomace of citrus germplasm at different maturity stages grown at different altitude

Germplasm	Altitude (m)	Peel IC_{50} (mg/ml)		Pomace IC_{50} (mg/ml)		Peel flavonoid (mg/QE/g)		Pomace flavonoid(mg/QE/g)	
		Mature green		Ripened		Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	1.53	1.24	3.14	2.12	141.33	138.00	158.33	157.00
	1000-1200	1.80	1.60	3.55	2.47	144.00	143.33	162.33	157.33
	1200-1400	1.97	1.62	3.59	2.89	145.00	143.80	168.33	162.00
	1400-1600	1.26	1.11	3.06	2.40	151.33	146.79	167.67	167.00
	>1600	1.72	1.48	3.83	2.50	156.00	148.34	159.25	154.33
Pommelo	**800-1000**	0.84	0.81	2.14	2.03	173.26	168.67	167.00	165.00
	1000-1200	1.29	1.02	1.78	1.70	180.00	178.67	173.33	170.00
	1200-1400	1.38	1.24	1.73	1.54	173.00	171.00	175.51	172.67
	1400-1600	1.44	1.43	1.97	1.60	177.02	173.33	194.33	190.67
	>1600	1.48	1.47	1.55	1.37	178.00	175.67	201.67	192.67
Rough lemon	**800-1000**	1.57	1.31	1.10	1.02	144.67	141.00	162.00	161.00
	1000-1200	1.54	1.26	1.13	0.93	148.33	117.12	168.75	163.00
	1200-1400	2.78	1.82	1.51	1.50	187.34	115.43	171.56	168.33
	1400-1600	1.93	1.48	1.70	1.00	179.85	161.18	162.00	159.67
	>1600	1.82	1.28	0.77	0.68	140.67	111.43	174.97	172.00

Factors	**CD (5%)**	**SE(d)**	**SE(m)**	**CD (5%)**	**SE(d)**	**SE(m)**	**CD (5%)**	**SE(d)**	**SE(m)**	**C.D(5%)**	**SE(d)**	**SE(m)**
Factor(G)	**0.06**	**0.03**	**0.02**	**0.09**	**0.04**	**0.03**	**0.89**	**0.45**	**0.31**	**1.17**	**0.58**	**0.41**
Factor(A)	**0.08**	**0.04**	**0.03**	**0.11**	**0.06**	**0.04**	**1.15**	**0.58**	**0.41**	**1.51**	**0.75**	**0.53**
Factor(S)	**0.05**	**0.02**	**0.02**	**0.07**	**0.04**	**0.02**	**0.73**	**0.36**	**0.26**	**0.95**	**0.48**	**0.34**
Interaction G XAXS	**0.20**	**0.10**	**0.07**	**0.27**	**0.14**	**0.09**	**2.82**	**1.41**	**0.99**	**3.69**	**1.85**	**1.31**

4.4. Essential oil and characterization

4.4.1. Oil yield (%)

The essential oil yield was invariably higher in the samples collected at mature green stage than the ripened one. It was also observed that the oil content was higher at the higher altitude (Table No. 4.2.9). The essential oil content was as low as 1.23 % in ripened pommelo collected from 800- 1000 m altitude and as high as 2.61 % in ripened rough lemon collected from > 1600 m altitude. In addition, ripened samples of Sikkim mandarin showed highest oil yield (2.40 %) at >1600 m altitude. Likewise, in pommelo it was 1.93 % and 2.61 % in rough lemon at >1600 m altitude

The samples collected at mature green stages showed higher oil yield than the ripened stage, which was estimated to the tune of 2.50 % Sikkim mandarin, >1600 m altitude, pommelo with 2.02 % in pommelo and 2.14 % in rough lemon in the samples collected at >1600 m altitude in all the cases.

All the germplasm showed increase in oil yield with increase in altitude irrespective of maturity stages.

4.4.2. Specific gravity

A maximum specific gravity (0.87) was calculated for pommelo samples collected at 1400-1600 m altitude at mature green stage (Table 4.2.9). The maximum specific gravity was found in Sikkim mandarin (0.86) in the samples collected at 1400-1600 m altitude, similar value (0.86) was obtained in the rough lemon samples collected from 800-1000 m and >1600 m altitude. Minimum values of specific gravity were 0.82, 0.84 and 0.84 respectively for Sikkim mandarin, pommelo and rough lemon collected at ripened stage from 800- 1000 m altitude range. As such there is no trend of increasing and decreasing with changes in altitude for all the germplasms.

4.4.3. Refractive Index (RI)

The highest refractive index of essential oil was in the range of 1.46 to 1.49 (Table 4.2.9). The mature green and ripening stage showed at par values in most of the cases. There was no specific trend in variations of refractive index with change in the altitude, maturity stage and the species.

4.4.4. Color value

Comparison of two stages in five different altitude amongst three germplasm showed pommelo in 1200-1400 m altitude at ripened stage as the best (1.30) amongst all the 30 combination in three-way analysis. However, it was found to be at par with rough lemon (1.26) at 1200-1400m at mature green stage and 1.26 in 1400-1600 m altitude in ripened stage (Table 4.2.9).

Pommelo has shown the general trend of increase in color value with increasing altitude, which was otherwise not seen for Sikkim mandarin and rough lemon. The color value was much higher for ripened fruit than mature green in case of pommelo and rough lemon. However, Sikkim mandarin showed the different pattern.

Table No 4.2.9: Characterization of essential oil of three different citrus germplasms of different altitude at different maturity stages

Germplasm	Altitude (m)	Oil yield (%)		Specific gravity		Refractive index (RI)		Color	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	1.49	1.48	0.85	0.84	1.46	1.46	0.69	0.81
	1000-1200	1.95	1.83	0.84	0.85	1.47	1.47	0.87	0.90
	1200-1400	1.97	1.84	0.82	0.86	1.48	1.47	1.04	0.95
	1400-1600	2.06	2.03	0.86	0.85	1.48	1.47	0.97	0.75
	>1600	2.50	2.40	0.82	0.86	1.49	1.48	1.06	0.73
Pommelo	800-1000	1.57	1.23	0.84	0.84	1.46	1.46	0.57	0.80
	1000-1200	1.49	1.43	0.86	0.85	1.47	1.47	0.68	0.89
	1200-1400	1.79	1.43	0.86	0.85	1.47	1.48	0.76	0.89
	1400-1600	1.60	1.50	0.87	0.82	1.48	1.49	0.92	1.01
	>1600	2.02	1.93	0.86	0.85	1.49	1.49	1.04	1.09
Rough lemon	800-1000	1.27	1.37	0.86	0.84	1.46	1.46	0.61	0.79
	1000-1200	1.41	1.54	0.83	0.85	1.47	1.47	0.67	0.80
	1200-1400	1.53	2.31	0.84	0.83	1.47	1.47	1.26	1.30
	1400-1600	1.64	2.50	0.85	0.82	1.48	1.48	0.22	1.26
	>1600	2.14	2.61	0.86	0.85	1.48	1.48	0.80	1.40

Factors	C.D (5%)	SE(d)	SE(m)	C.D (5%)	SE(d)	SE(m)	C.D (5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)
Factor(G)	0.11	0.06	0.04	0.01	0.003	0.002	0.004	0.002	0.001	0.05	0.03	0.02
Factor(A)	0.14	0.07	0.05	0.01	0.003	0.002	0.005	0.003	0.002	0.07	0.03	0.02
Factor(S)	0.09	0.05	0.03	0.004	0.002	0.002	0.003	0.002	0.001	0.04	0.02	0.02
Interaction G X AX S	0.35	0.17	0.12	0.02	0.009	0.006	0.013	0.006	0.004	0.17	0.08	0.06

4.4.5. Free fatty acid (%)

Rough lemon was found to have maximum free fatty acid (FFA) i.e. 8.27 % in the sample collected from 1400-1600 m with at par value of 7.60 % and 7.77 % in the sample collected from 1200-1400 m altitude respectively at ripened and mature green stage. There was no significant difference amongst some of the treatments as illustrated at Table 4.2.10.

Lowest value of all the interaction was found in the Pommelo sample collected from 800-1000 m (2.26 %) at ripened stage and the highest record was 7.38 % in the samples collected from >1600 m altitude at mature green. Sikkim mandarin was recorded with 7.83 % (at mature green stage, 1200-1400 m altitude) as the highest and lowest was 5.07 % (ripened stage, 800-1000 m altitude). There was increasing pattern in regards to FFA for pommelo fruit with increasing altitude. However, no such pattern was found for Sikkim mandarin and rough lemon.

The FFA content was also found to be higher for mature green stage fruit than ripened stage irrespective of the germplasms and stages. It was also noted that rough lemon showed the highest FFA followed by Sikkim mandarin and pommelo.

4.4.6. Peroxide value (meq. O_2/Kg oil)

The three-way interpretation peroxide value was in the range of 11.00 meq. O_2/Kg oil in the essential oil of pommelo collected from 800-1000 m altitude at ripened stage to 31.33 meq. O_2/kg oil in Sikkim mandarin collected from 1200-1400 m altitude at mature green stage (Table 4.2.10). There was increase trend of peroxide value with increasing altitude for Sikkim mandarin. However, pommelo and rough lemon were not following such trend. The comparison between stages revealed mature green stage being much higher peroxide value than the ripened stage.

Moreover, altitude and stages interaction showed >1600 m altitude with the highest peroxide value at mature green stage and that of 800-1000 m altitude in ripened stage with the lowest

4.4.7. Antioxidant activity by DPPH method (%)

Pommelo collected from >1600 m was recorded with highest antioxidant activity (DPPH method) i.e. 82 % followed by 81.71% in rough lemon at 1400-1600 m altitude at ripened stage. Whereas, the lowest antioxidant activity (DPPH method) was 65.67 % in Sikkim mandarin collected at mature green stage. As such the pattern of increase in antioxidant activity (DPPH method) was not in line with increasing altitude for all the germplasms. The comparison of two stages revealed mature green stage much higher for antioxidant activity (DPPH method) than the ripened stage (Table 4.2.10).

4.4.8. Phenol (g/kg)

The pommelo samples collected from 1400-1600 m altitude at ripened stage had highest phenol (82.67 g/kg) content in the extracted essential oil. In mature stage samples, phenol was even higher to the tune of 89.59 g/kg in rough lemon collected from1400-1600 m (Table 4.2.10). Sikkim mandarin however had maximum phenol (81.92 g/kg) in the essential oil extracted from the samples collected at >1600 m altitude.

There was increasing trend in phenol content with increasing altitude for pommelo both in ripened and mature green stage. However, no such trend was found for Sikkim mandarin and rough lemon. Moreover, ripened stage was found to have higher phenol content than mature green stage for all the germplasm.

Table No 4.2.10: Chemical characterization of essential oil of three different citrus germplasms of different altitude at different maturity stages

Germplasm	Altitude (m)	Free fatty acid (%)		Peroxide value (meq. O_2/kg oil)			Antioxidant activity by DPPH method (%)			Phenol (mg/g)		
		Mature green	Ripened	Mature green	Ripened		Mature green	Ripened		Mature green	Ripened	
Sikkim mandarin	**800-1000**	6.77	5.10	15.00	11.67		73.67	65.67		7.97	7.91	
	1000-1200	6.21	5.47	23.00	15.40		80.53	74.85		8.08	8.12	
	1200-1400	7.83	5.04	30.00	18.50		68.51	68.30		8.09	8.08	
	1400-1600	5.74	5.38	30.67	23.67		73.00	71.26		8.04	8.08	
	>1600	5.54	5.50	31.33	27.33		75.99	75.04		8.16	8.19	
Pommelo	**800-1000**	2.57	2.26	12.00	11.00		77.68	72.99		7.97	7.98	
	1000-1200	2.87	2.61	11.33	10.80		78.08	71.95		8.07	8.12	
	1200-1400	3.17	3.14	13.96	12.99		70.99	70.76		8.09	8.13	
	1400-1600	5.86	5.77	16.03	15.67		81.37	68.77		8.12	8.19	
	>1600	7.38	6.01	17.68	17.04		82.00	77.84		8.15	8.27	
Rough lemon	**800-1000**	5.80	5.11	15.33	11.33		69.67	69.00		7.98	8.06	
	1000-1200	6.40	6.03	12.33	11.67		71.72	66.00		8.04	8.75	
	1200-1400	7.77	7.60	14.67	13.00		80.95	79.68		8.04	8.53	
	1400-1600	8.27	7.60	17.33	13.67		81.71	70.82		8.05	8.60	
	>1600	7.90	5.23	14.67	13.33		72.43	71.60		8.14	8.96	
Factors	**C.D(5%)**	**SE(d)**	**SE(m)**	**C.D (5%)**	**SE(d)**	**SE(m)**	**C.D (5%)**	**SE(d)**	**SE(m)**	**C.D (5%)**	**SE(d)**	**SE(m)**
Factor(G)	**0.23**	**0.12**	**0.08**	0.39	0.19	0.14	1.68	0.85	0.60	0.46	0.23	0.16
Factor(A)	**0.30**	**0.15**	**0.11**	0.51	0.25	0.18	2.19	1.09	0.77	0.59	0.29	0.21
Factor(S)	**0.19**	**0.10**	**0.07**	0.32	0.16	0.11	1.37	0.69	0.49	0.37	0.19	0.13
Interaction G X AXS	**0.74**	**0.37**	**0.26**	1.24	0.62	0.44	5.37	2.68	1.89	1.45	0.72	0.51

4.4.9. Ester value (mg KOH/ g of oil)

The ester value of the extracted essential oil was highest in the samples of Sikkim mandarin (Table 4.2.11) which was to the tune of 157.54 mg KOH/g oil in the samples collected from 1200-1400 m altitude at ripened stage. It was found to be statistically at par with 156.39 mg KOH/g oil in rough lemon at 1000-1200 m altitude in ripened stage (Table 4.2.9). Likewise, pommelo collected from 1200-1400 m altitude at ripened stage showed maximum ester value of oil (153.86 mg KOH/g oil) which was at par with pommelo collected from 1000-1200 m altitude at ripened stage (151.81mg KOH/g oil). Further in rough lemon, the highest was 170.10 mg KOH/ g oil in the samples collected from >1600 m altitude at ripened stage and was followed by 156.39 mg KOH/g of oil at 1000-1200 m altitude at ripened stage.

The ester value was not found to be affected with the altitude. In addition, ripened stage showed higher ester value than mature green stage irrespective of germplasms and altitude.

4.4.7. Saponification value (mg KOH/g of oil)

The saponification value was recorded up to 165.35 mg KOH/g oil in essential oil of rough lemon collected at 1000-1200 m altitude in ripened stage which was at par with that of Sikkim mandarin i.e. 163.67 mg KOH/g oil at 1200-1400 m altitude in ripened stage (Table 4.2.11). Essential oil extracted from Pommelo peel had 161 mg KOH/g oil saponification value in the samples collected from 1200-1400 m altitude at ripened stage. The lowest saponification value was found in essential oil for pommelo (130 mg KOH/g oil) collected from 800-1000 m altitude in mature green stage. There was no pattern of increase in saponification value with respect to increase in altitude for all the germplasms. Furthermore, ripened stage showed comparatively higher saponification value than mature green stage irrespective of the three germplasms and two stages.

4.4.6. Acid value (%)

Acid value of the essential oil was found to be maximum in mature green pommelo fruits (12.74 %) collected from 1400-1600 m altitude followed by the mature green samples collected at >1600 m altitude (11.67 %). In rough lemon maximum acid value of essential oil was 8.97 % in the sample collected from 1000-1200 m altitude at ripened stage. Sikkim mandarin had highest value of 8.37 % collected from 1400-1600 m altitude in mature green stage (Table 4.2.11).

There was increasing trend for acid value in pommelo with increasing altitude. However, no such trend was observed for Sikkim mandarin and rough lemon. The acid value was found to be significantly higher for mature green fruit than ripened one.

4.4.10. Iodine value (g I_2/100g)

Iodine value of the essential oil was up to 116.33 g/I_2/100g in mature green Sikkim mandarin samples collected from 1400-1600 m altitude with at par value of 115.67 g/I_2/100g (1000-1200 m altitude in mature green stage) and 116 g/I_2/100g (1400-1600m altitude in ripened stage) as mentioned in Table 4.2.11.

Whereas, in pommelo, 112.67 g/I_2/100g was found to be the maximum value in the samples collected from 1400-1600 m altitude at ripened stage, followed by 104.45 g I_2/100g (1000-1200 m altitude at ripened stage). In rough lemon, 115.67 g

I100g was found to be the highest value as followed by 111.01 gI_2/100g (1000-1200 m altitude in mature green stage).

Amongst the gemplasm, Sikkim mandarin was found to be with highest iodine value followed by pommelo and rough lemon. Whereas the lowest iodine value was 74 g I_2/100g in rough lemon at 1200-1400 m altitude in ripened stage.

4.4.11. Thiocyanogen value (%)

Out of all 30 combinations, pommelo oil extracted from the samples collected from >1600 m altitude at ripened stage was found to be having maximum thiocyanogen value (77.87 %) with at par value of 77.09 % (>1600 m altitude in mature green stage) and 76.75 % (1400-1600 m altitude at ripened stage), 76.09 % (1400-1600 m altitude at mature green stage, 75.86 % (1200-1400 m altitude at ripened stage), 75.84 % (1200-1400 m altitude at mature green stage) and 75.63 % (1000-1200 m altitude at ripened stage) in pommelo (Table 4.2.11).

Sikkim mandarin oil recorded thiocyanogen value up to 77.30 % at 1400-1600 m altitude at mature green stage as the highest with at par value of 75.10 % (1000-1200 m altitude at mature green stage),75.29 % (1200-1400 m altitude at ripened stage), 75.17 % (1200-1400 m altitude at mature green stage), 74.96 % (1400-1600 m) altitude at ripened stage, 75.40 % (>1600 m altitude at ripened stage) and 74.53 % (>1600 m altitude at mature green stage).

In rough lemon, 75.78 % was the highest value (>1600 m altitude at ripened stage) with at par value of 73.73 % (800-1000 m altitude at mature green stage) and 73.33 % (1400-1600 m altitude at ripened stage).

There was increasing trend in thiocyanogen value with increasing altitude in case of pommelo. However, no such trend was observed for Sikkim mandarin and rough lemon. The thiocyanogen value was much higher for mature green fruit than ripened stage.

Germplasm	Altitude (m)	Ester value (mg KOH/ g oil)		Saponification value (mg KOH/ g oil)		Acid Value (%)		Iodine value (g I_2/100 g oil)		Thiocyanogen value (%)	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	133.16	145.03	141.33	152.67	8.17	7.63	102.00	102.00	70.00	68.33
	1000-1200	140.13	140.40	146.00	145.67	5.87	5.27	115.67	106.46	75.10	73.63
	1200-1400	150.93	157.54	156.67	163.67	5.74	6.12	110.67	109.96	75.17	75.29
	1400-1600	132.63	136.20	141.00	142.00	8.37	5.80	116.33	116.00	77.30	74.96
	>1600	145.67	153.04	154.00	161.34	8.33	8.30	114.64	113.33	75.40	74.53
Pommelo	**800-1000**	122.07	130.60	130.00	136.68	7.93	6.08	104.00	101.77	74.96	71.46
	1000-1200	146.50	151.81	155.00	158.01	8.51	6.20	104.29	104.45	75.63	74.91
	1200-1400	149.43	153.86	159.67	161.00	10.23	7.14	93.39	78.38	75.86	75.84
	1400-1600	125.93	129.52	137.34	138.67	11.67	7.82	112.67	92.00	76.75	76.09
	>1600	119.33	127.22	131.00	137.33	12.74	10.11	105.62	93.06	77.87	77.09
Rough lemon	**800-1000**	129.30	148.23	135.67	155.00	6.37	6.77	103.33	94.76	73.73	72.33
	1000-1200	147.06	156.39	155.00	165.35	7.94	8.97	111.01	92.73	74.73	72.42
	1200-1400	141.63	145.38	149.67	153.34	8.04	7.96	94.00	74.00	72.79	68.33
	1400-1600	141.57	146.04	150.00	153.34	8.43	7.31	115.67	106.67	73.33	69.67
	>1600	135.33	170.10	143.67	177.34	8.34	7.25	86.41	104.67	75.78	67.67

Factors	C.D (5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)	C.D (5%)	SE(d)	SE(m)	C.D(5%)	SE(d)	SE(m)	C.D (5%)	SE(d)	SE(m)
Factor(G)	**1.31**	**0.66**	**0.46**	**1.22**	**0.61**	**0.43**	**0.35**	**0.17**	**0.12**	**1.16**	**0.58**	**0.41**	**0.89**	**0.45**	**0.32**
Factor(A)	**1.69**	**0.85**	**0.59**	**1.58**	**0.78**	**0.56**	**0.45**	**0.22**	**0.16**	**1.50**	**0.75**	**0.53**	**1.15**	**0.58**	**0.41**
Factor(S)	**1.07**	**0.54**	**0.38**	**0.99**	**0.49**	**0.35**	**0.28**	**0.14**	**0.10**	**0.95**	**0.47**	**0.33**	**0.74**	**0.36**	**0.26**
Interaction (G XAXS)	**4.15**	**2.08**	**1.47**	**3.86**	**1.93**	**1.36**	**1.10**	**0.55**	**0.39**	**3.67**	**1.84**	**1.29**	**2.83**	**1.41**	**0.99**

Table No 4.2.11. Chemical characterization of essential oil of three citrus germplasms of different altitude at different maturity stages

4.5. GCMS analysis of essential oil at different maturity stages

4.5.1. Mature green stage

4.5.1.1. 800-1000 m altitude

In Sikkim mandarin, 40 components were determined (Table 4.3.1) which showed 100.01 % of the total oil. Limonene (87.93 %) being the highest followed by γ-terpinene (5.37 %), myrcene (1.61 %), linalool (1.52 %), α-pinene (0.65 %), β-pinene (0.36 %), α–terpinolene (0.26 %), α-terpineol (0.27%) and carvacrol (0.20 %). Besides that, other component is found in trace form. In addition, pommelo in 800-1000 m altitude showed 33 components were identified with 100.05 % of the total oil (Table 4.3.2).

Limonene showed 94.00 % being the highest. Myrcene (1.49 %), β-pinene (1.36 %), Germacrene D (0.54 %), α-pinene (0.42 %), sabinene (0.33 %), γ-terpinene (0.27 %), linalool (0.19 %), α-terpineol (0.11 %), β-ocimene (0.07 %), terpinen-4-ol (0.06 %), germacrene B (0.03 %) and octanal (0.01 %).

In rough lemon samples collected from 800-1000 m showed a total of 40 components represenitng 97.79 % of the total oil. Limonene was found to be major component (83.26 %). In addition, sabinene (3.30 %), myrcene (1.39 %), terpinen-4-ol (1.19 %), linalool (0.58 %), α-sinensal (0.69 %), β-sinensal (0.65 %), trans-carveol (0.31 %), allo-ocimenol (1.22 %), nerolidyl acetate (0.72 %) ,cyclopentan-1,2-dione (0.67 %), p-mentha-2,8-diene, 1-hydroperoxide (0.61 %) ,β-bisabolene (0.32 %) ,β-farnesene (0.33 %) were the major identified compounds.(Table 4.3.3).

4.5.1.2. 1000-1200 m altitude

At the altitude range of 1000-1200 m (Table 4.3.1), peel oil of Sikkim mandarin had 42 components (100.71 % of thetotal oil) amongst which, limonene accounted to 89.56 % followed by myrcene (1.56%), γ-terpinene : 1.43 % ,linalool: 0.92 %, sabinene (0.69 %), β-pinene:0.48 %, Germacrene D: 0.45 %, α-pinene:0.38 %, terpinen-4-ol :0.37 % , α-terpineol:0.30 %, α-sinensal: 0.23 %, α-farnsesene:0.26 %, carvone:0.20 %, cis-linalool oxide:0.35 % and trans-linaool oxide:0.23 % .

In Table 4.3.2, Pommelo peel oil was also having 38 constituent compounds with 104.10 % of the total oil and the number of these compounds was found to be highest at this altitude. Amongst all, limonene share was 95.27 % followed by β-pinene (2.99 %), sabinene (0.53 %), α–pinene (0.49 %), myrcene (1.44 %), β-ocimene (0.21 %),linalool (0.40 %), γ-terpinene (0.21 %), β-ocimene (0.21 %), α-terpineol (0.16 %), Germacrene D (0.13 %), terpinen-4-ol (0.08 %) and octanal (0.04 %).

Rough lemon peel oil was recorded with 50 components with 100.18 % of the total oil. Some of the major compounds identified were limonene (80.64 %), γ -terpinene (11.07 5), β -pinene (2.18 %), myrcene (1.50 %) α -pinene (0.85 %) and α-terpinolene (0.57 %).

4.5.1.3. 1200-1400 m altitude

At this altitudinal range, Sikkim mandarin peel oil was detected with 34 components (100.01 % of the total oil) as elaborated in Table 4.3.1. Limonene content was highest (83.69 %) followed by γ -terpinene: 6.45 %, myrcene: 1.69 %, sabinene (1.55 %), linalool (2.05 %), 4-terpineol :0.44 %, thymol (0.41%), terpinolene (0.31 %), α-terpineol (0.25 %) and α-pinene (0.95 %).

Likewise, pommelo peel oil had 41 constituent compounds representing 103.02 % of the total oil. Limonene was the major constituents (91.62 %) followed by myrcene (1.59 %), β-pinene (2.68 %), Germacrene D (0.72 %), γ-terpinene (0.68 %), α–pinene (0.46 %), sabinene (0.59 %), linalool (0.21%), β-ocimene (0.32 %), α-terpineol (0.12 %), terpinen-4-ol (0.09 %), decanal (0.02 %), germacrene B(0.02 %) and octanal (0.01%) as shown in Table 4.3.2 .

A total of 38 compounds were identified with 94.50 % of total oil in rough lemon (Table 4.3.3). Amongst them some of the major compounds were limonene (81.17 %), myrcene (1.48 %), 1-octanol (6.84 %), p-cymene (0.46 %), nonanal (0.46 %), α-pinene (0.88 %) and β-pinene (0.53 %).

4.5.1.4. 1400-1600 m altitude

Sikkim mandarin in this altitude showed 33 number of components was identified with 100.62 % of total oil content (Table 4.3.1). Limonene was found to be 84.13 % with the highest content in oil. Likewise, γ -terpinene:6.37 %, linalool:2.28 %, myrcene: 1.60 %, α -pinene: 0.90 %, sabinene :0.75 %, β-pinene: 0.55 %, hordenine:0.54 %, terpinen-4-ol:0.42 %, α-terpineol: 0.24 % were found. In addition, other compounds were showing very least oil percentage.

Likewise, pommelo was found with 36 components were found in this altitude which showed 99.94 % of the total oil. Limonene (92.37 %) showed the highest followed by β-pinene (2.28 %), myrcene (1.47 %), Germacrene D (0.90 %), α-pinene (0.43 %), linalool (0.28%), α-terpineol (0.14 %), γ-terpinene (0.17 %), terpinen-4-ol (0.09%), β-ocimene (0.09%), Germacrene B (0.03 %), octanal (0.03 %) and decanal (0.02 %) as mentioned in Table 4.3.2.

In rough lemon total of 41 compounds were found which occupies 100.01 % of total oil. Limonene content was the highest (91.62 %) followed by sabinene (0.59 %), β-pinene (2.67 %), myrcene (1.59 %), γ-terpinene (0.68 %) and Germacrene-D (0.72 %) (Table 4.3.3).

4.5.1.5. >1600 m altitude

Sikkim mandarin in this altitude showed 35 components with 99.39 % of total oil content. Limonene (89.06 %) holds the highest. In addition, γ-terpinene (4.76 %), β-pinene: 1.65 %, linalool: 1.09 %, α -pinene: 0.71 %, α-fenchene: 0.62 %, sabinene: 0.41 %, myrcene: 0.27 %, α -terpinolene: 0.21 % is found. Likewise, other components were found in trace forms (Table 4.3.1).

Likewise, pommelo was observed with 40 components as illustrated in Table 4.3.2 which holds 100.18 % of the total oil. Limonene (93.22 %) showed the maximum followed by β-pinene (1.82 %), myrcene (1.53 %), α-pinene (0.41 %), sabinene (0.36 %), α-terpineol (0.11%), linalool (0.15 %), γ-terpinene (0.09 %), β-ocimene (0.18 %), terpinen-4-ol (0.05 %), octanal (0.02 %), decanal (0.02 %) and rough lemon oil was recorded with 36 compounds which holds 100.02 % of the total oil. The major compound was limonene (87.86 %), β-phellandrene (5.77 %), myrcene (1.98 %), α-terpineol (0.68 %), decanal (0.25 %), α -pinene (0.54 %), β-pinene (0.34 %), β-ocimene (0.34 %) and γ-terpinene (0.31 %) as illustrated in Table 4.3.3.

4.5.2. Ripened stage

The chemical composition of mandarin peel essential oil was illustrated in table 4.3.1.

4.5.2.1 800-1000 m altitude

GC- MS analysis of the extracted oil presented in Table No 4.3.1 revealed the 49 identified compounds in Sikkim mandarin peel with 100.68 % of the total oil. Limonene (88.39 %) holds the highest share followed by γ-terpinene (4.64 %), linalool (1.46 %), myrcene (1.65 %),α–pinene (0.78 %), sabinene (0.50 %), β-pinene(0.55 %), octanal (0.42 %), α–terpinolene (0.18 %), terpinen-4-ol (0.12 %), α-terpineol (0.15 %), decanal (0.16 %), o-cymen-5-ol (0.12 %) and so on.

In pommelo fruits collected from altitude 800-1000 m showed 47 identified compounds, limonene being the major component with 94.10 % of oil yield followed by myrcene (1.61 %), α-pinene (0.36 %), β-pinene (0.22 %), γ-terpinene (0.25 %), linalool (0.17 %), α-terpineol (0.11 %), Germacrene D (0.14 %), terpinen-4-ol (0.05 %), β-ocimene (0.04 %) and octanal (0.02 %) (Table 4.3.2)

Whereas rough lemon was found with 51 components with 99.96 % of total oil was detected (Table 4.3.3). Limonene showed 75.67 % of total oil. α -pinene (0.64 %), β-pinene (0.86 %), myrcene (1.20 %), p-cymene (4.95 %), γ-terpinene (3.30 %), linalool (0.78 %), citronellal (1.41 %), carvacryl methyl ether (3.80 %), ar- tumerone (1.17 %), neoisopulegol hydrate (0.50 %).

4.5.2.2 1000-1200 m altitude

A total of fifty-seven compounds were identified in an essential oil content of Sikkim mandarin representing 104.36 % of the total oil produced. Amongst the components, limonene was estimated to be 87.64 % followed by γ-terpinene: 4.31 %, myrcene-1.53 %, linalool: 1.90 %, Sabinene: 1.13 %, α-pinene: 0.52 %, β-pinene oxide: 0.75 %, octanal: 0.28 % (Table 4.3.1).

Pommelo oil sample was found with 48 constituent compounds in this altitude (1000-1200 m). Limonene showed 91.16 %. followed by myrcene with 1.50 %, sabinene (0.37 %), α-Pinene (0.41 %), β-pinene (0.95 %), linaool (0.17 %), α-terpineol (0.17 %), Germacrene D (0.13 %), terpinen-4-ol (0.10 %), decanal (0.05%), β-ocimene (0.03 %), γ-terpinene (0.02 %), octanal (0.02%) (Table 4.3.2).

Rough lemon showed 52 compounds with 141.61 % of the total oil. The major compounds found were limonene (92.56 %), sabinene (1.05 %), β-pinene (5 %), p-cymene (11.24 %), linalool (1.54 %), terpinen-4-ol (1.18 %), α-terpineol (2.25 %), myrcene (0.92 %), octanal (0.62 %), γ-terpinene (0.59 %), neoisopulegol hydrate (1.73 %), limonene glycol (2.91 %), α-thujone (1.04 %), 2-cyclohexen-1-one (0.98 %), 2-hydroxy-1,8 cineole (1.76 %) as mentioned in Table 4.3.3.

4.5.2.3 1200-1400 m altitude

The total 68 compounds were identified in the essential oil with 101.56 % of the total oil (Table 4.3.1). Main constituents in sikkim mandarin were limonene (87.20 %), γ-Terpinene: 4.82 %, linalool: 1.50 %, myrcene: 1.57 %, thymol methyl ether: 0.58 %, α -terpineol: 0.23 %, octanal: 0.32 %, sabinene: 0.50 % and α -pinene: 0.62 % and other compounds showed trace amount of oil (Table 4.3.9).

Likewise, in pommelo oil (Table 4.3.2) at 1200-1400 m altitude 34 constituent compound were identified. Limonene being the major one with 82.38 %. The next was myrcene (1.25 %), β-pinene (0.76 %), linalool (0.89 %), Germacrene D (0.41 %), octanal (0.24%), γ-Terpinene (0.33 %), α–pinene (0.31%), octanal (0.24 %), sabinene (0.23 %) and decanal (0.12 %).

In rough lemon oil ,52 components were detected which occupies 99.92 % of the total oil. Limonene (81.89 %), β-pinene (0.44 %), myrcene (1.84 %), linalool (0.89 %), terpinen-4-ol (0.95 %), α-terpineol (0.87%), β-phellandrene (8.77 %) and α-bisabolol (0.22 %) were the major constituents (Table 4.3.3).

4.5.2.4 1400-1600 m altitude

At the altitude range of 1400-1600 m, 47 compounds were identified which occupies 100.91 % of the total oil in Sikkim mandarin as mentioned in (Table 4.3.1) with 101.02 % of the total oil. Limonene (73.09 %) holds the highest content in oil. Other constituent compounds were linalool (9.14 %), γ-terpinene (4.46 %), terpinen-4-ol (1.81 %), myrcene (1.44%), α -terpineol (1.33 %), thymol (1.22 %), octanal (0.68 %), β-pinene (0.72%), sabinene (0.67 %), α-pinene (0.60 %), α-muurolol (0.47 %), bornyl acetate (0.34 %), carvacryl methyl ether (0.28 %), decanal (0.24 %).

In pommelo, 37 components were recorded (Table 4.3.2). Limonene (89.70 %) holds the highest share followed by lianool (1.38 %), α-terpineol (0.79 %), γ-terpinene (0.34 %), octanal (0.24 %) and β-pinene (0.23 %).

Further rough lemon was noticed with 44 components with 100.83 % of total oil yield were found. Limonene (91.65 %), α-pinene (0.70 %), sabinene (0.15 %), β -pinene (0.48 %), myrcene (1.40 %), octanal (0.49 %), γ-terpinene (0.94 %), linalool (1.95%) which was mentioned in Table 4.3.3.

4.5.2.5 >1600 m altitude

A total of 54 compounds (100.95 % of the total oil) were found in the oil content of Sikkim mandarin with highest share of limonene (88.46 %). Other prominent compounds were γ-terpinene (4.00 %), linalool (1.67 %), myrcene (1.61 %), sabinene (0.70 %) and α-pinene (0.69 %) as shown in (Table 4.3.1)

At >1600 m pommelo was recorded with 40 compounds in total with highest share of limonene (80.89 %). Other compounds were β-pinene (1.14 %), myrcene (0.63 %), α-pinene (0.41 %), α-terpineol (0.33 %), sabinene (0.29%), linalool (0.22 %) and γ-terpinene (0.09 %) as illustrated in Table 4.3.2.

In rough lemon showed 52 components with 99.15 % of total oil were identified in this altitude. Limonene (87.48 %), α-pinene (0.58 %), β-pinene (5.22 %), terpinen-4-ol (0.62 %), linalool (0.25 %), α-terpineol (0.22 %) and γ -terpinene (0.21 %) were found as mentioned in Table 4.3.3.

Table No 4.3.1: GC-MS profile of the area (%) of essential oil of *Citrus reticulata* peel at different maturity stages

Compound name	Altitude range (m)									
	800-1000		1000-1200		1200-1400		1400-1600		>1600	
	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
α-Thujene	0.12	0.16	-	0.10	0.22	0.13	0.21	0.14	0.15	0.14
α -Pinene	0.66	0.78	0.38	0.53	0.96	0.62	0.90	0.60	0.71	0.70
Camphene	-	0.00	-	0.01	-	0.03	-	0.19	-	0.01
Sabinene	0.18	0.50	0.69	1.13	1.55	0.50	0.75	0.67	0.41	0.70
β -Pinene	0.36	0.55	0.48	0.76	0.58	0.41	0.55	0.72	1.65	0.44
Myrcene	1.62	1.65	1.56	1.53	1.69	1.57	1.60	1.44	0.27	1.61
Octanal	0.12	0.42	0.20	0.28	0.27	0.33	0.27	0.68	0.08	0.35
(+)-2-Carene		0.06		0.08	-	-	-	-	-	0.02
Limonene	87.93	88.39	89.46	87.64	83.70	87.20	84.13	73.09	89.06	88.46
β -Ocimene	0.04	0.04	0.05	0.14	0.16	0.08	0.14	0.11	0.06	0.07
γ -Terpinene	5.37	4.64	1.43	4.32	6.45	4.82	6.37	4.46	4.76	4.00
Acetophenone		0.02	-	-	-	-	-	-	-	-
1-Octanol	0.06	0.07	-	0.21	-	0.10	0.05	0.21	0.04	0.16
α -Terpinolene	0.26	0.18	-	-	0.05	0.24	0.30	0.26	0.21	0.19
α -p-dimethylstyrene		0.01	-	-	-	0.03	-	-	-	0.01
Linalool	1.52	1.46	0.92	1.90	2.05	1.50	2.28	9.14	1.09	1.67
Nonanal	0.04	0.02	-	0.04	-	0.03	-	-	0.02	
Trans-p-Mentha-2,8-dienol	-	0.01	-	-	-	0.01	-	0.04	-	0.01
Cis-p-Mentha-2,8-dien-1-ol	-	0.01	-	-	-	0.02	-	0.04	-	-
Citronellal	0.01	0.05	0.03	0.10	0.05	0.10	0.03	0.06	0.04	0.08
Terpinen-4-ol	0.22	0.12	0.37	0.17	0.44	0.19	0.42	1.81	0.12	0.16
α -Terpineol	0.27	0.15	0.30	0.18	0.25	0.23	0.24	1.33	0.12	0.21

Decanal	0.19	0.16	0.10	0.17	0.13	0.18	0.15	0.24	0.13	0.23
Nerol		0.01		-		0.01		-		0.01
Carvacryl methyl ether		0.07		-		-		0.28		-
Carvacrol	0.21	0.21	-	-	-	0.17	-	-		0.07
Neral	0.01	0.03		0.02	0.03	0.03		0.07	0.02	0.01
Carvone		0.01	0.20	0.20		0.03		0.05		0.02
Geraniol	0.01	-	0.19	-		0.02	0.02	0.12		-
Geranial	0.03	0.03		-		-	0.03	0.14	0.02	0.03
Perillaldehyde	0.05	0.04	0.06	-	0.03	0.05	0.04	0.11	0.02	0.05
o-Cymen-5-ol		0.13		-		-		-		-
Undecanal	0.01	0.01		-		0.02		-		0.05
δ-Elemene	0.03	0.03		0.03		0.04	0.03	-	0.03	0.01
β -Elemene	0.02	0.02		0.02	0.02	0.02	-	-	0.01	0.01
Dodecanal		0.02		0.02	0.02	0.02	0.02	-	0.01	0.02
Germacrene B		0.01		0.02		0.04		-		0.01
Trans-β -Farnesene		0.02		-		0.04		-		-
Germacrene D		0.05	0.45	0.05		0.07		0.11		0.04
Trans- α -Farnesene		0.01		0.02		0.02		-		0.02
γ -Elemene		0.03		-	0.01	0.01	0.02	-		0.02
Terpinolene		-		0.19		-		-		-
Thymol methyl ether	0.09	-		0.14		0.58	0.14	-	0.10	0.14
α -Citral		-	0.08	0.03		-		-		-
2-Decyn-1-ol		-		0.03		-		-		-
Thymol		-		0.09	0.41	0.41		1.22		0.19
Bicyclogermacrene		-		0.02	0.08	0.08		-		-
α -Terpinene	0.11	-	0.04	-	-	0.09	0.16	0.07	-	
Trans-Carveol		-		-		0.02		-		-
Bornyl acetate		-		-		0.05		0.34		-
Geranyl acetate		-		-		0.01		-		-

Caryophyllene		-		-		0.02		-		-
Cis- α -Bergamotene		-		-	0.05	0.03		-		-
Trans- β-Bergamotene		-		-		0.01		-		-
α-Bisabolene		-		-		0.01		-		-
δ-Cadinol	-	-	-	-	-	0.07	-	-	-	-
α-Bisabolol	-	-	-	-	-	0.03	-	0.15	-	-
α -Humulene	-	-	-	-	-	-	-	0.11	-	-
Phenylacetaldehyde	-	-	-	-	-	-	-	0.02	-	-
Cis-citral	-	-	-	-	0.05	0.05	-	-	-	-
(Z) Sabinene Hydrate	-	-	-	-	-	-	-	0.02	-	-
β -Terpineol	-	-	-	-	-	0.02	-	0.04	-	-
Borneol	-	-	-	-	-	-	-	0.10	-	-
p-Cymen-9-ol	-	-	-	-	-	-	-	0.10	-	-
2,6-Octadien-1-ol	0.02	-	0.16	0.16	-	0.05	-	0.10	-	-
Limonen-10-ol	-	-	-	-	-	-	-	0.05	-	-
1,3-Dioxolane, 2,2-dimethyl-4,5-dipropenyl	-	-	-	-	-	-	-	0.03	-	-
Cyclohexene	-	-	-	-	-	-	-	0.11	-	-
Neoisopulegol hydrate	-	-	-	-	-	-	-	0.04	-	-
Limonene glycol	-	-	-	-	-	-	-	0.13	-	-
Carvotanacetone <8-hydroxy>	-	-	-	-	-	-	-	0.04	-	-
2-Cyclohexen-1-one	-	-	0.06	-	-	-	-	0.04	-	-
2 Methyl isoborneol	-	-	-	-	-	-	-	0.09	-	-
β -Bisabolene	-	-	-	-	-	0.04	-	0.08	-	-
α -Elemol	-	-	-	-	-	-	-	0.03	-	-
Spathulenol	-	-	-	-	-	-	-	0.02	-	-
trans-Valerenyl acetate	-	-	-	-	-	-	-	0.02	-	-

α -Muurolol	-	-	-	-	-	-	-	0.47	-	-
Cadin-4-en-10-ol	-	-	-	-	-	-	-	0.07	-	-
Zierone	-	-	-	-	-	-	-	0.14	-	-
γ -Terpinyl acetate	-	-	-	-	-	-	-	-	-	0.06
Cis-Limonene oxide	-	-	-	-	-	-	-	-	0.01	0.01
Trans-Limonene oxide	-	-	-	-	-	-	-	-	0.01	0.02
1- Nonanal	0.02		-		-		-		-	-
1-Nonanol		-	-	-	0.05	0.05	-	-	-	0.01
m-Cymen-8-ol	-	-	-	-	-	-	-	-	-	0.01
Trans- α-Bergamotene	-	-	-	-	-	0.05	-	-	-	0.03
Dodecanal	0.03	0.03	-	-	-	0.02	-	0.02	-	0.01
γ -Elemene	0.01	0.01	-	-	-	0.01	-	0.02	0.02	0.02
α - Farnesene	0.02		0.26	-	-		-	-	0.01	
β -Farnesene	0.03	0.03	-	0.02	0.04	0.04	0.04	0.04	0.02	0.02
Germacrene D	0.06	0.06	-	0.45	0.07	0.07	0.09	0.09	0.04	0.04
α -Farnesene	-	0.02	-	0.26		-	0.07	0.07	-	0.01
α -bergamotol	-	-	-	-	0.12	0.12	-	-	-	-
Hordenine	-	-	-	-	-	-	0.54	0.54	-	-
δ-Cadinene	0.01	0.01	-	-	-	-	-	-	-	-
Germacrene B	0.04	0.04	0.03	0.03	0.01	0.01	0.07	0.07	0.04	0.04
β-Sinensal	0.04	0.04	0.30	0.30	0.05	0.05	0.05	0.05	-	0.02
α-Sinensal	0.05	0.05	0.23	0.23	-	0.07	0.07	0.07	0.01	0.01
p-Cymene	-	-	0.15	0.15	0.09	0.09	0.22	0.22		-
α -Fenchene	-	-		-	-	-	0.62	-		0.62
Ascaridole	-	-		-	-	-	-	-	0.02	0.02
Ethanone	-	-	0.03	0.03	-	-	-	-	-	-
Cis- Linalool oxide	-	-	0.35	0.35	-	-	-	0.06	-	-
Trans-Linaool oxide	-	-	0.23	0.23	-	-	-	-	-	-
trans-p-Mentha-2,8-	-	-	0.11	0.11	-	-	-	-	-	-

dienol										
Cyclopentan-1,2-dione	-	-	0.07	0.07	-	-	-	-	-	-
Cis-p-Mentha-2,8-dien-1-ol	-	-	0.12	0.12	-	-	-	-	-	-
(-)-trans-Isopiperitenol	-	-	0.09	0.09	-	-	-	-	-	-
Trans-carveol	0.01	-	0.26	0.26	-	-	-	0.03	-	-
Cis-Carveol	-	-	0.14	0.14	-	-	-	-	-	-
2-Cyclohexen-1-one	-	-		0.06	-	-	-	-	-	-
p-Mentha-2,8-diene, 1-hydroperoxide	-	-	0.25	0.25	-	-	-	-	-	-
Nerolidol	-	-	0.15	0.15	-	-	-	-	0.01	0.01
Limonene-1,2-diol	-	-	0.50	0.50	-	-	-	-		-
Carvacrol	-	0.21		-	-	0.17	-	-	0.07	0.07
Spathulenol	-	-	0.07	0.07	-	-	-	-	-	-
Cadin-4-en-10-ol	-	-	0.02	0.02	-	-	-	-	-	-
Cis Thijopsenic acid	-	-	0.05	0.05	-	-	-	-	-	-
Torulosol	-	-	0.09	0.09	-	-	-	-	-	-
γ -Terpinolene	-	-	-	-	0.31	0.31	-	-	-	-
Cosmene	-	-	-	-	0.01	0.01	-	-	-	-
β -eudesmol	-	-	-	-	0.01	0.01	-	-	-	-
γ -Terpineol acetate	0.13		-		-		-		-	
Total	**100.01**	**100.68**	**100.71**	**104.36**	**100.01**	**101.56**	**100.62**	**101.02**	**99.39**	**100.95**

Table No 4.3.2: GC-MS profile of the area (%) of Pommelo (*Citrus maxima*) peel essential oil in different maturity stages

Compound name	Altitude range (m)									
	800-1000		1000-1200		1200-1400		1400-1600		>1600	
	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
α-Thujene	0.02	0.02	0.02	0.01	0.03	-	0.02	0.13	0.08	0.02
α -Pinene	0.42	0.36	0.49	0.41	0.46	0.31	0.43	-	0.41	0.41
Sabinene	0.33	0.12	0.53	0.37	0.59	0.23	-	-	0.36	0.29
β -Pinene	1.36	0.22	2.99	0.95	2.68	0.76	2.28	0.23	1.82	1.14
Myrcene	1.49	1.61	1.44	1.50	1.59	1.25	1.47	-	1.53	0.63
Octanal	0.01	0.02	0.04	0.02	0.01	0.24	0.03	0.24	0.02	-
Limonene	94.00	94.10	95.27	94.81	91.62	82.38	92.37	89.70	93.22	80.89
β -Ocimene	0.07	0.04	0.21	0.03	0.32	-	0.09	-	0.18	-
γ -Terpinene	0.27	0.25	0.24	0.02	0.68	0.33	0.17	0.34	0.09	0.09
Cis-Linalool oxide	0.03	0.03	0.06	-	0.04	-	0.05	2.98	0.08	0.16
Trans- Linalool oxide		-	0.03	0.03		0.12	0.03	1.86	0.03	0.07
α -Terpinolene		0.03		-		-		-		-
p-Cymenene		0.03		-		0.49		1.12		-
Linalool	0.19	0.17	0.40	0.17	0.21	0.89	0.28	1.38	0.15	0.22
Trans limonene oxide					0.02					
Cis-Limonene oxide	0.04	0.13	0.12	0.22		-		-	0.04	0.64
Trans-p-Mentha-2,8-dienol	0.03	0.10	0.07	0.13		0.45	0.04	2.40	0.03	0.47
Cis-p-Mentha-2,8-dien-1-ol	0.05	0.16	0.14	0.25		0.25	0.05	2.29	0.05	1.37
Terpinen-4-ol	0.06	0.05	0.08	0.10	0.09	-	0.09	-	0.05	-
Trans-Chrysanthenyl acetate		0.01		-		-		-		-
α -Terpineol	0.11	0.11	0.16	0.17	0.12	-	0.14	0.79	0.11	0.33
(-)-trans-		0.06		-		0.18		0.83		-

Isopiperitenol										
(-)-cis-Isopiperitenol		-	0.04	0.08		-	0.04	-		-
Thymol methyl ether		0.27		-		0.77		-		-
Carvacryl methyl ether					0.09				0.03	
Cis-Carveol	0.04	0.12	0.10	0.19		-	0.05	-	0.11	-
Trans-Carveol	0.05	0.17	0.15	0.29		-	0.09	-	0.07	-
Carveol									0.05	
cyclohexane									0.12	
Geraniol	0.07	0.03	0.05	0.13	0.05	0.09		-	0.03	-
Geranial	0.03	0.05	0.07	0.15	0.06	-		-	0.09	-
Perillaldehyde		0.02		0.04	-	0.13		-		0.18
o-Cymen-5-ol		0.02		-	-	-		-		-
p-Mentha-2,8-diene, 1-hydroperoxide	0.12	0.20	0.09	0.35	-	0.47	0.02	-	0.11	0.98
p-mentha-1,8-diene-4-hydroperoxide					-		-	-	0.06	
Tricyclo[3.1.0.0(2,4)]Hexane		0.19		-	-	-	-	-	-	-
(+)-Nerolidol		0.11		-	-	-	0.12	-	-	0.22
Limonene glycol		0.03	0.02	-	-	2.04		11.75	-	1.30
Bicyclo[3.1.1]heptane		0.06		-	-	-		-	-	-
α -Sinensal	0.12	0.20	0.16	0.35	0.01	0.62	0.11	-	-	1.35
β -Sinensal		-	0.16	0.36		-		-	0.16	0.94
Geranyl acetate		0.31		0.97		-		-		-
Limonen-10-yl-acetate		0.04		-	0.02	-	0.03	-	0.03	-
β -Caryophyllene		0.02		-		-		-	-	-
Trans- α-		0.02		-		-		-	-	-

Bergamotene										
Germacrene D	0.54	0.14	0.13	0.13	0.72	0.41	0.90	-	0.62	-
Germacrene B	0.03		-	-	0.02		0.03	-	-	-
β -Bisabolene	-	0.02		-	-	-	-	-	-	-
Spathulenol	-	0.02	0.05	0.04	-	-	0.04	-	-	-
(Z) Asarone	-	0.07		-	-	-	-	7.82	-	-
β-Caryophyllene oxide	-	0.04	0.03	0.06	-	-	-	-	0.03	0.16
2-Furanmenthanol	-	-	-	0.05	-	-	-	-	-	-
Decanal	-	-	-	0.05	0.02	0.12	-	-	-	-
Sabina ketone	-	-	-	0.07	-	-	-	-	-	-
Neryl acetate	-		-	-	0.02	-	-	-	-	
Geranyl acetate	-		-	-	0.09	-	0.09	-	0.02	
Heptyl acetate	-	-	-	0.03	-	-	-	-	-	-
Nerol	0.05	-	-	0.07	-	-	-	-	-	-
Nerolidol	0.07		-	-	-		-		-	
Citronellal	-	0.03	-	0.07	0.02	0.41	-	-	-	-
Citronellol	-	-	-	0.06			-	-	-	-
Neral	-	-	0.04	0.08	0.04	0.27	-	-	0.06	-
Carvone	0.05	0.18	0.12	0.28	-	0.95	0.08	2.62	0.05	1.84
Limonen-10-ol	-	-	0.07	0.04	-	-	-	-	-	0.08
Cosmene	-	-		-	-	-	-	-	-	0.06
β Cyclohomogeraniol	-	-		-	-	-	-	-	-	0.06
(+)-Limonene oxide	-	-		-	-	-	-	-	-	0.04
α –Campholenic aldehyde	-	-		-	-	-	-	-	-	0.05
α -Terpinyl acetate	-	-		-	-	-	-	-	-	0.05
Isospathulenol	-	-		-	-	-	-	-	0.02	0.04
Nootkatol	-	-		-	-	-	-	-	-	0.04
α -Cyperone	-	-		-	-	-	-	-	-	0.04

Perilla alcohol	-	-	-	0.04	-	-		-	-	0.16
β -Farnesene	-	-	-	0.17	-	-	0.06	-	-	-
α-Farnesene	-	-	-	-	-	-	-	-	-	0.68
Citronellyl acetate	-	-	-	0.13	-	-	-	-	-	-
3-Heptadecen-5-yne, (Z)-	-	-	-	0.07	-	-	-	-	-	-
Caryophyllene	-	-	-	0.04	0.03	-	-	-	0.07	-
β -Copaene	-	-	0.04	0.03	-	-	-	-	0.03	-
Furfuryl alcohol,tetra hydro-α,α,5- trimethyl -5-vinyl	-	-		-	-	0.06	-	-	-	-
(Z)Salvene	-	-		-	-	0.89	-	-	-	-
β -Thujone	-	-		-	-	0.13	-	1.43	-	0.16
Cyclooctanone	-	-		-	-	0.32	-	-	-	-
2-Cyclohexen-1-ol	-	-	0.18	-	-	0.44	-	0.43	0.02	0.27
Bicyclo [3.3.0]Oct-2-en-7-one	-	0.01	-	-	-	0.41	-	-	-	0.12
Bicyclo[2.2.2]oct-2-ene	-		-		-		0.02		-	
Isothujol	-	-	-	-	-	0.12		-	-	-
bicyclogermacrene	-		-		0.05		0.03		-	
α-Campholenic aldehyde	-	-	-	-	-	-		-	-	0.05
α -Terpinyl acetate	-	-	-	-	-	-		-	-	0.05
Isospathulenol	-	-	-	-	-	-	0.03	-	-	0.04
Nootkatol	-	-	-	-	0.06	-	0.03	-	-	0.04
α -Cyperone	-	-	-	-	-	-	-	-	-	0.04
(R)-lavandulyl acetate	-	-	-	-	-	0.08	-	-	-	-
Lavundulol	-		0.06		-	-	-		-	

α-Limonene diepoxide	-	-	-	-	-	0.10	-	2.56	-	-
(E) Apritone	-	-	-	-	-	0.30	-	-	-	-
Neo-Isopulegol hydrate	-	-	-	0.05	-	0.18	-	-	-	-
Trans-Verbenol	-	-	-	-	-	0.55	-	-	-	-
Bicyclo[3.2.0]heptan-2-one	-	-	-	-	-	0.10	-	1.65	-	
2,6-Octadien-1-ol	-	-	-	-	0.03	0.42	0.02	-	0.02	-
α -Terpinyl acetate	-	-	-	-	-	0.24	-	-	-	-
β -Geraniolene	-	-	-	-	-	-	-	0.68	-	-
Seudenone	-	-	-	-	-	-	-	0.83	-	-
Cyclopentan-1,2-dione	-	-	-	-	-	-	-	4.89	-	-
Trans-Carveol	-	0.09	-	0.09	-	0.95	-	2.77	-	1.70
Cis-carveol	-	-	-	-	-	0.45	-	1.53	-	0.78
Arthole	-	-	-	-	-	-	-	0.94	-	-
7-Oxa bicyclo(4.1.0) heptane	-	-	-	-	-	-	-	1.94	-	-
Trans-Ascaridol glycol	-	-	-	-	-	-	-	0.97	-	-
2-Methyl isoborneol	-	-	-	-	1.01	-	-	-	-	-
Uroterpenol	-	-	-	-	1.14	-	-	-	-	-
Orcinol, monoacetate	-	-	-	-	0.75	-	-	-	-	-
Cis-Citral	-	-	-	-	-	-	-	-	-	0.15
Santolina triene	-	-	-	-	-	-	-	-	-	0.21
Isoborneol	-	-	-	-	0.10	-	-	0.10	-	0.15
Isopiperitenone	-	-	-	-	-	-	-	-	-	0.12
Perillyl alcohol	-	-	-	-	-	-	-	-	-	0.06
α-Selinene	-	-	-	-	0.08	-	-	0.08	-	0.91
Geranyl acetate	0.14	-	-	-	-	-	-	-	-	-
γ -Cadinene	0.02	-	-	-	0.02	-	-	-	-	-

2R,4R)-p-Mentha-6,8-diene, 2-hydro peroxide	0.11	-	-	-	-	-	-	-	-	-
Benzene, 1-eth yl-2,3-dimethyl	0.10	-	-	-	-	-	-	-	-	-
α -Fenchene	0.01	-	0.01	0.01	-	0.11	-	-	-	-
Patchenol		-	0.06	-	-	-	-	-	0.04	
3-Hydroperoxy-3 methyl-6-cyclohex-1-ene	-	-	0.18	-	-	-	-	-	-	-
α -phellandrene	-	-	-	-	0.03	-	-	-	-	-
β -Phellandrene	-	-	-	-		-	0.44		-	-
camphene	-	-	-	-	0.01	-	-	-	0.09	
p-cymene	-	-	-	-	0.02	-	-	-	-	-
δ-Elemene	-	-	-	-	0.05	-	-	-	-	-
β -Elemene	-	-	-	-	0.02	-	0.16	-	-	-
γ -Elemene	0.02	-	-	-	-	-	0.01	-	-	-
Total	**100.05**	**100.08**	**104.10**	**103.76**	**103.02**	**100.01**	**99.94**	**148.70**	**100.18**	**99.85**

Table No 4.3.3: GC-MS profile of the area (%) of essential oil of *Citrus jambhiri* peel at different maturity stage

Compound name	Altitude (m)									
	800-1000		1000-1200		1200-1400		1400-1600		>1600	
	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
α-Thujene	0.03	0.20	0.23	0.13	0.19	0.04	0.03	0.16	0.03	0.03
α -Pinene	0.39	0.64	0.85	0.62	0.88	0.49	0.46	0.70	0.54	0.58
Sabinene	3.30	0.27	0.27	1.05	0.53	-	0.59	0.15		0.01
β -Pinene	0.34	0.86	2.18	5.00	0.01	0.44	2.67	0.48	0.34	5.22
Myrcene	1.39	1.20	1.50	0.92	1.48	1.84	1.59	1.40	1.98	0.34
Octanal	0.12	0.16	0.09	0.62	-	0.12	0.01	0.49	0.12	1.86
Octanol	0.08	-	-	-	-	-	-	-	-	
p-Cymene	-	4.95		11.24	0.46	0.04	0.02	-	-	0.09
Limonene	83.26	75.67	80.64	92.56	81.17	81.89	91.62	91.65	87.86	87.48
2-ethyl butanol	-	-	-	-	0.08	-	-	-	-	-
β -Ocimene	0.07	0.12	0.14	-	0.05	0.15	0.32	0.06	0.34	0.12
γ -Terpinene	0.42	3.30	11.07	0.59	0.25	0.36	0.68	0.94	0.31	0.21
α -Terpinene	-	-	-	-	-	-	-	-	0.13	
Camphene	-	-	-	-	-	0.09	0.01	-	-	-
1-Octanol	-	0.08	0.07	-	6.84	0.23		0.08	-	
α-Terpinolene	-	0.23	0.57	-	0.03	0.05	0.06	0.09	0.07	0.05
α-p-dimethyl styrene		0.20	-	0.10	-	-		-		-
Linalool	0.58	0.78	-	1.54	0.18	0.89	0.21	1.95	0.31	0.25
Nonanal	0.06	0.07	0.28	0.24	0.46	0.06	-	0.04	0.04	-
Nonanol	-	-	-	-		0.07	-	-	-	-
Trans-p-Mentha-2,8-dien-1-ol	-	0.04	-	0.15	-	0.03	-	0.05	-	0.05
Hexadec-(9Z)-enal	-	0.06	-	-	-	-	-	-	-	-
Cis-p-Mentha-2,8-dien-1-ol	-	0.07	-	0.24	-	0.04	-	0.09	-	0.08
Isopulegol 2	-	0.04	-	-	-	-	-	-	-	-
Citronellal	0.06	1.41	-	0.70	0.08	0.08	0.02	0.06	0.02	0.10
Citronellol	-	-	-	0.66	0.32	0.13	-	-		0.03
Terpinen-4-ol	1.19	0.23	0.24	1.18	0.03	0.95	0.09	0.09	0.08	0.62
p-Cymen-9-ol	-	0.08		-	0.23	-		0.02		-
α -Terpineol	0.40	0.39	0.44	2.25	0.02	0.87	0.12	0.13	0.68	0.22
γ -Terpineol	-	-	0.04	-	-	-	-			
Santolina triene	-	0.10	-	-	-	-	-	0.02		0.02
Decanal	0.07	0.15	-	0.26	-	0.07	0.02	0.17	0.25	0.06

- = compounds not reported

4.6. Analysis of pectin

4.6.1. Pectin Qualitative test

The qualitative characteristic of pectin is summarized in Table 4.4.1.

4.6.1.1. Color

The color of pectin extracted from all the germplasm were dark brown in color at 800-1000 m and >1600 m altitude in Sikkim mandarin as illustrated in Table 4.4.1. However, pommelo and rough lemon were dark brown color in 800-1000 m, 1400-1600 m and >1600 m altitude. Whereas the remaining altitude showed light brown color.

4.6.1.2. Solubility in cold and hot water

Pectin solution was found to be insoluble in cold water. Whereas mixture get dissolved in hot water in all the stages and germplasm (Table 4.4.1).

4.6.1.3. Solubility in cold and hot alkali

Pectin solution was found to be insoluble in cold alkali with yellow precipitates but in hot alkali mixture gets dissolves in all the germplasm and stages as mentioned in Table 4.4.1.

4.6.1.4. Sugar and organic acid (%)

Three factor analysis of variance is shown in Table No. 4.4.1. Sikkim mandarin was found to be superior having highest sugar and organic acid up to (56.53 %) at an altitude range of >1600 m at ripened stage. It was found to be at par with 55.33 % and 56.33 % at 1400-1600 m and >1600 m altitude in mature green stage.

In Pommelo, sugar and organic acid was recorded up to 66.67 % (1400-1600 m) altitude in mature green stage which was also found to be at par with 63.93 % and 65.67 % at >1600 m altitude in ripened and mature green stage.

Whereas in rough lemon it was 43.67 % at altitude range 800-1000 m. It was found to be at par with 43.33 % (800-1000 m) in ripened stage, 1400-1600 m altitude in mature green stage, 43 % (>1600 m) altitude in ripened stage and 42.33 % (>1600 m) altitude in mature greens stage.

Table No 4.4.1: Estimation of pectin content and its qualitative analysis at different altitudinal range

Germplasm	Altitude range (m)	Color		Solubility of dry pectin in cold water		Solubility of dry pectin in hot water		Solubility of dry pectin in cold alkali		Solubility of dry pectin in hot alkali	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	Dark brown	Dark brown	Insoluble	Insoluble	Mixture dissolves	Mixture dissolves	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	Dissolves	Dissolves
	1000-1200	Light brown	Light brown	Insoluble	Insoluble	Mixture dissolves	Mixture dissolves	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	Dissolves	Dissolves
	1200-1400	Light brown	Light brown	Insoluble	Insoluble	Mixture dissolves	Mixture dissolves	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	Dissolves	Dissolves
	1400-1600	Light brown	Light brown	Insoluble	Insoluble	Mixture dissolves	Mixture dissolves	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	Dissolves	Dissolves
	>1600	Dark brown	Dark brown	Insoluble	Insoluble	Mixture dissolves	Mixture dissolves	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	Dissolves	Dissolves
Pommelo	800-1000	Dark brown	Dark brown	insoluble	Insoluble	Mixture dissolves	Mixture dissolves	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	Dissolves	Dissolves
	1000-1200	Light brown	Light brown	Insoluble	Insoluble	Dissolves with water color	Sparingly dissolves	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	Dissolves	Dissolves
	1200-1400	Light brown	Light brown	Insoluble	Insoluble	Mixture dissolves with water	Sparingly dissolves in yellow color	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	Dissolves with yellow color	Dissolves with yellow color

						color					
	1400-1600	Dark brown	Dark brown	Dissolves	Insoluble	Mixture dissolves	Sparingly dissolves in yellow color	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	dissolves with yellow color	dissolves with yellow color
	>1600	Dark brown	Dark brown	sparingly dissolves	Insoluble	sparingly dissolves	mixture dissolves	Insoluble (forms yellow precipitates)	Insoluble (forms yellow precipitates)	dissolves with yellow color	dissolves with yellow color
Rough lemon	**800-1000**	Dark brown	Dark brown	insoluble	Insoluble	Dissolves with transparent color	mixture dissolves	insoluble	insoluble	dissolves with yellow color	dissolves with yellow precipitate
	1000-1200	Light brown	Light brown	insoluble	Insoluble	Dissolves	Dissolves with pale yellow precipitates	insoluble	insoluble	dissolves with yellow color	dissolves with transparent color
	1200-1400	Light brown	Light brown	insoluble	Insoluble	Mixture dissolves	Dissolves with transparent color	insoluble	insoluble	dissolves with yellow color	dissolves with yellow precipitate
	1400-1600	Dark brown	Dark brown	insoluble	Insoluble	Mixture dissolves	Sparingly dissolves in yellow color	insoluble	insoluble	Dissolves with yellow color	dissolves with yellow color
	>1600	Dark Brown	Dark brown	insoluble	Insoluble	Sparingly dissolves	mixture dissolves	insoluble	insoluble	dissolves with yellow color	dissolves

4.6.2. Pectin Quantitative test

4.6.2.1. % Yield (Residual peel)

The % yield of fruit pectin was found to be highest (40.82 %) at the mature green stage of Sikkim mandarin grown at an altitude of >1600 m compared to other germplasms grown at different altitudes. Rough lemon had the highest % yield on wet basis to the tune of 37.19 % at >1600 m altitude followed by pommelo i.e. 18.33 % collected from (>1600 m altitude) at mature green stage. Lowest % yield was 10.67 % at ripened pommelo fruit collected from 800-1000 m altitude. The % yield was comparatively higher for mature fruit than that of ripened stage fruit irrespective of the altitude and stages. There was also increasing trend in % yield with increasing altitude for all the germplasm (Table 4.4.2)

4.6.2.2. Equivalent weight (g/mol)

The equivalent weight content in pectin was highest in Sikkim mandarin collected from > 1600 m (496.33 g/mol) at ripened stage which is statistically at par with 494.67 g/mol at 1400-1600 m altitude in ripened stage as mentioned in Table 4.4.2.

In mature green stage, highest pectin equivalent weight (492.67 g/mol) was estimated in the pommelo samples collected from >1600 m and the lowest was in the samples of pommelo (338.33 g/mol) collected from 800-1000 m altitude.

On the other hand, pommelo (494.33 g/mol) showed the highest value at >1600 m altitude in ripened stage which was found to be at par with 492.67g/mol (1400-1600 m altitude) and 492.07 g/mol (>1600 m altitude) in mature green stage.

Moreover, rough lemon (492.67 g/mol) at >1600 m altitude in mature green stage was recorded with maximum equivalent weight which was found to be at par with 481.33 g/mol in >1600 m altitude at ripened stage.

There was pattern of increase in equivalent weight with respect to increasing altitude in all the germplasms. Pectin equivalent weight was found to be highest for ripened fruit than the mature green stage irrespective of the germplasms and altitude.

4.6.2.3. Methoxyl content (%)

Rough lemon fruit samples collected from > 1600 m altitude was recorded with highest pectin methoxyl content (7.31 %) at ripened stage, and the value is highest amongst all other germplasms collected from different altitude as compared to rest of the germplasm illustrated in Table 4.4.2.

Highest methoxyl content in Sikkim mandarin was recorded in the samples collected from > 1600 m altitude at ripened stage (7.01 %). In pommelo, the highest value was seen to be 7.17 % in the ripened samples collected from >1600 m altitude. The lowest record was however, 4.32 % for mature green fruit samples of Sikkim mandarin collected at 800-1000 m altitude.

There was increasing trend for Sikkim mandarin and rough lemon with increasing altitude. However, no such pattern was found in pommelo. In all the cases, ripened fruit pectin had higher methoxyl content the mature green stage irrespective of germplasms and altitude.

Table No. 4.4.2: Yield, equivalent weight and methoxyl content in pectin of citrus germplasms at different altitude in different maturity stages

Germplasm	Altitude (m)	% yield (Residual peel)		Equivalent weight (g/mol)			Methoxyl content (%)		
		Mature green	Ripened	Mature green	Ripened		Mature green	Ripened	
Sikkim mandarin	800-1000	29.49	24.33	385.89	393.33		4.32	5.07	
	1000-1200	35.83	28.33	395.00	412.89		5.33	6.29	
	1200-1400	36.82	31.00	396.33	451.81		6.57	6.92	
	1400-1600	37.00	33.00	414.89	494.67		6.64	6.93	
	>1600	40.82	34.67	444.49	496.33		6.93	7.01	
Pommelo	800-1000	13.00	10.67	338.33	408.28		6.27	6.80	
	1000-1200	15.00	11.33	362.87	420.39		6.73	6.83	
	1200-1400	16.67	11.07	430.01	446.69		5.43	5.90	
	1400-1600	16.00	11.33	450.18	492.02		5.87	7.02	
	>1600	17.33	16.00	492.67	494.33		7.11	7.17	
Rough lemon	800-1000	18.19	16.33	370.26	370.67		4.70	5.63	
	1000-1200	25.31	21.00	387.00	410.28		4.99	6.30	
	1200-1400	26.17	22.00	414.41	417.56		6.00	6.28	
	1400-1600	35.02	27.33	461.67	475.67		6.50	6.59	
	>1600	37.19	27.67	481.33	492.67		6.53	7.31	
Factors	**C.D (5%)**	**SE(d)**	**SE (m)**	**C.D (5%)**	**SE (d)**	**SE (m)**	**C.D(5%)**	**SE (d)**	**SE (m)**
Factor (G)	**0.10**	**0.50**	**0.35**	**3.64**	**1.79**	**1.27**	**0.15**	**0.08**	**0.06**
Factor (A)	**0.13**	**0.65**	**0.46**	**4.63**	**2.31**	**1.64**	**0.20**	**0.10**	**0.07**
Factor (S)	**0.08**	**0.41**	**0.29**	**2.97**	**1.46**	**1.04**	**0.13**	**0.06**	**0.05**
Interaction G X AXS	**0.31**	**1.58**	**1.12**	**11.34**	**5.67**	**4.01**	**0.49**	**0.25**	**0.17**

4.6.2.4. Anhydrounic acid (%)

The highest anhydrounic acid (AUA) content was recorded in pectin of ripened rough lemon followed by pommelo and Sikkim mandarin. Rough lemon grown at >1600 m altitude was recorded with 43.75 % AUA and at par with (43.69 %) in pommelo at >1600 m attitude in mature green stage, 43.54 % (pommelo) at 1400-1600 m altitude in mature green stage, 43.27 % (Sikkim mandarin) at >1600 m altitude in mature green stage and 43.20 % (Sikkim mandarin) at 1400-1600 m altitude in mature green stage as shown in Table 4.4.3.

Furthermore, pommelo was recorded with 43.69 % (>1600 m altitude) in ripened stage being next highest after Sikkim mandarin, at par values were recorded to be 42.74 % (1000-1200 m altitude) 42.79 % (1200-1400 m altitude), 43.54 % (1400-1600 m altitude) in ripened stage as well as 42.42 % (1200-1400 m), 42.61 % (1400-1600 m altitude) and 42.73 % (>1600 m altitude) in mature green stage in pommelo fruit. In rough lemon, highest AUA content was 43.75 % (collected from >1600 m altitude) in ripened stage. It was at par with 42.58 % (1200-1400 m) and 43.20 % (1400-1600 m altitude) in ripened stage.

The increase in pectin AUA was in uniformity with increasing altitude for Sikkim mandarin and pommelo. However, no such pattern was observed for rough lemon. Likewise, pectin AUA was also found much higher for ripened fruit than the mature green stage irrespective of altitude and germplasm.

4.6.2.5. Degree of Esterification (%)

The pectin of mature green pectin samples of Sikkim mandarin collected from >1600 m altitude accounted for highest degree of esterification (91.79 %) which was found to be at par with 90.75 % (1400-1600 m altitude) in mature green stage, 90.34 % (1400-1600 m altitude) in ripened stage and 90.94 % (>1600 m altitude) in ripened stage in Sikkim mandarin mentioned in Table 4.4.3.

On the other hand, highest DE in pommelo was 90.93 % (1400-1600 m altitude) which was in turn at par with 90.56 % (1400-1600 m altitude) in mature green stage, 90.64 % (>1600 m altitude) in ripened stage and 90.79 % (>1600 m altitude) in mature green stage.

Moreover, rough lemon was found with 82.60 % as the highest DE at >1600 m altitude in mature green stage which was followed by 79.73 % (>1600 m altitude) in ripened stage.

Whereas the lowest was 55.35 % (800-1000 m altitude) in ripened stage for rough lemon out of all the 30 combination.

There was trend of increase in pectin DE with respect to increase in altitude for all the germplasm. The mature green stage also showed much higher DE as compared to ripened stage irrespective of all the germplasm and two stages.

4.6.2.6. Ash content (%)

The data regarding ash content recorded up to 8.83 % in (pommelo) at (>1600 m) altitude in ripened stage as the highest ash content and was in turn at par with 7.82 % (>1600 m altitude) in mature green stage. While the lowest value of 4.04 % was recorded in rough lemon in (1000-1200 m altitude) at mature green stage. However, in rough lemon (7.93 %) at (>1600 m altitude) in ripened stage was found with highest ash content.

In addition, Sikkim mandarin showed 7.92 % (>1600 m altitude) in ripened stage as the highest value which was at par with 7.37 % (1400-1600 m altitude) in ripened stage shown in Table 4.4.3.

The data revealed that Sikkim mandarin at ripened stage recorded increase in ash content with increase in altitude. Whereas the rest germplasm donot follow the trend. it was also noted that ripened stage had higher ash content than mature green stage.

Table No. 4.4.3: Chemical characterization of pectin of citrus germplasms at different altitude in different maturity stages

Germplasm	Altitude (m)	AUA (%)		DE (%)			Ash content (%)		
		Mature green	Ripened	Mature green	Ripened		Mature green	Ripened	
Sikkim mandarin	800-1000	37.67	41.95	88.24	85.67		4.43	5.17	
	1000-1200	41.65	42.03	88.90	88.65		6.06	6.09	
	1200-1400	41.86	42.58	89.33	89.23		6.55	6.66	
	1400-1600	42.31	43.20	90.75	90.34		6.50	7.37	
	>1600	43.27	43.75	91.79	90.94		6.92	7.92	
Pommelo	800-1000	38.19	42.19	85.00	82.33		6.02	6.20	
	1000-1200	41.96	42.74	85.94	85.00		5.59	5.93	
	1200-1400	42.42	42.79	89.65	87.77		5.77	6.13	
	1400-1600	42.61	43.54	90.79	90.56		6.46	7.60	
	>1600	42.73	43.69	90.93	90.64		7.82	8.83	
Rough lemon	800-1000	36.54	41.07	58.33	55.35		4.17	4.25	
	1000-1200	29.00	42.51	59.00	58.50		4.04	4.20	
	1200-1400	26.29	42.79	70.73	60.67		4.50	5.10	
	1400-1600	26.59	42.71	77.70	77.67		6.60	6.60	
	>1600	35.24	43.75	82.60	79.73		6.80	7.93	
Factors	**C.D (5%)**	**SE(d)**	**SE (m)**	**C.D (5%)**	**SE (d)**	**SE (m)**	**C.D (5%)**	**SE (d)**	**SE (m)**
Factor (G)	**0.41**	**0.21**	**0.15**	**0.58**	**0.29**	**0.21**	**0.25**	**0.13**	**0.09**
Factor (A)	**0.53**	**0.27**	**0.19**	**0.75**	**0.38**	**0.27**	**0.33**	**0.16**	**0.12**
Factor (S)	**0.34**	**0.17**	**0.12**	**0.47**	**0.24**	**0.17**	**0.21**	**0.10**	**0.07**
Interaction G X AXS	**1.31**	**0.65**	**0.46**	**1.85**	**0.92**	**0.65**	**0.80**	**0.40**	**0.28**

4.6.2.7. FTIR in Mature green stage

The FTIR in mature green stage was shown in Table 4.4.4(representative FITR spectra can be seen at Fig.4.6.2.7) which showed Sikkim mandarin in matured green stage was observed with 3364, 3585 and 3604 cm^{-1} at O-H bond in the fruit collected at 800-1000 m, 1200-1400 m and >1600 m altitudes. Likewise, 2949, 2921, 3048, 2952 and 2957 cm^{-1} were found as C-H stretch in 800-1000 m, 1000-1200 m, 1200-1400 m, 1400-1600 m and >1600 m.

In addition, 1727, 1718, 1714, 1717 and 1696 cm^{-1} C=O bond were found in 800-1000 m, 1000-1200 m,1200-1400 m, 1400-1600 m and >1600 m. C-N stretch was also noted in matured green stages which showed 1026, (1222, 1076), (1229, 1075, 1018) (1990 ,1339, 1206, 1074), (1075 ,1019) cm^{-1} in 800-1000 m, 1000-1200 m, 1200-1400 m, 1400-1600 m and >1600 m altitude.

On the other hand, pommelo was found with sharp and strong peak as O-H stretch with maximum 3596 cm^{-1} frequency at 1200-1400 m altitude followed by 3552 cm^{-1} (>1600 m) altitude. Likewise, C-H stretch with (Carboxylic acid) showed 3046 cm^{-1} and 3098 cm^{-1} as lowest and highest at 1200-1400 m and >1600 m altitude. In regard of C-H stretch i.e. alkane showed 2955 cm^{-1} as the highest at >1600 m altitude. C= O stretch having α, β unsaturated ester was found with 1723 cm^{-1}(s), 1689 cm^{-1}(s) at 800-1000 m altitude, 1720 cm^{-1}(s) (1200-1400 m), 1716 cm^{-1} (1400-1600 m altitude) and 1707 cm^{-1} (>1600 m altitude). C-N stretch which is aliphatic amine showed highest 1401 cm^{-1}(m) 1061 cm^{-1}(m) at 800-1000 m altitude.

Besides that, there is C-O stretch with 1368 cm^{-1} frequency at 1400-1600 m altitude as the maximum. C-Cl stretch with alkyl halides showed the 856 cm^{-1} frequency (1000-1200 m altitude). C-H out of plane blend which accounts for aromatics showed the least 792 cm^{-1} (800-1000 m) altitude. Further N-H bend was noted in pommelo (1621 cm^{-1}) at 1400-1600 m altitude.

Likewise, rough lemon in mature green stage showed O-H stretch was found at 3569 (1000-1200 m), 3401 cm^{-1} (1200-1400 m). Further, C=O at 1710 cm^{-1} (800-1000 m), 1717 cm^{-1} (1000-1200 m), 1694 cm^{-1} (1200-1400 m), 1723 cm^{-1} (1400-1600 m) and 1716 cm^{-1} (>1600 m). Likewise, C-H stretch in 2954 cm^{-1} (800-1000 m); 3062 cm^{-1}, 2992 cm^{-1} (1000-1200 m); 2950 cm^{-1} , 1191 cm^{-1} (1200-1400 m), (1400-1600 m) and 3058 cm^{-1}, 2959 cm^{-1} (>1600 m).C-N stretch in 1072 cm^{-1} (800-1000 m); 1223 cm^{-1},1074 cm^{-1}, 1020 cm^{-1} (1000-1200 m) ; 1403 cm^{-1}, 1219 cm^{-1} (1400-1600 m) and 1074 cm^{-1} (>1600 m).C-Cl stretch in 800-1000 m (855 cm^{-1}) ; 866 cm^{-1}, 830 cm^{-1} (1400-1600 m).

4.6.2.8. FTIR in ripened stage

The FTIR spectrum of Sikkim mandarin with corresponding functional groups are mentioned in Table 4.4.4 (representative FITR spectra can be seen at Fig.4.6.2.8). In ripened stage, it showed sharp and strong peaks at 3607, 3135, 3537 cm^{-1} at 800-1000 m, 1000-1200 m and 1200-1400 m as O-H stretch. Likewise, C-H stretch in the frequency of 2956 cm^{-1}, 2939 cm^{-1}, 2957cm^{-1}, 3048 cm^{-1} and 2959 cm^{-1} were observed in the sample collected from all the altitudes. Similarly, C=O bond was found at 1727cm^{-1},1721cm^{-1}, 1721cm^{-1}, 1712 cm^{-1} and 1715cm^{-1} at 800-1000 m, 1000-1200 m, 1200-1400 m, 1400-1600 m and >1600 m and >1600 m which showed α, β unsaturated ester. C-N stretch with aliphatic 1075, 1201 at 800-1000 m.

Likewise, 1073, 1219 (1000-1200 m). Further (1074 cm^{-1}, 1214 cm^{-1}); (1017cm^{-1}, 1064 cm^{-1}) and (1021cm^{-1}, 1095 cm^{-1}) were observed in 1200-1400 m, 1400-1600 m and >1600 m altitudes accordingly. In addition, alkyl halides (C-Cl) were noted only in 1400-1600 m (883 cm^{-1}) and C-H wag in 1400-1600 m (1103 cm^{-1}) and >1600 m (1108 cm^{-1}) was observed.

Likewise, in pommelo, the FTIR analysis in ripened stage at 800-1000 m showed 3618 cm^{-1}, and 3012 cm^{-1} frequency as sharp and strong peaks at O-H stretch. In addition, 3503 $cm^{-1,}$ frequency (1000-1200 m) was found. While the least was 3034 cm^{-1} (1400-1600 m) altitude. Further (>1600 m) holds 3349 cm-1 as the O-H stretch.

On the other hand, C-H stretch (alkane) showed 3098 cm^{-1} (1000-1200 m, >1600 m) as the highest in ripened and mature green stage and that of 2921 cm^{-1} (1400-1600 m) as the least in mature green stage.

Further, C= O stretch with α, β unsaturated ester was found with 1723 cm^{-1}(s) frequency was found to be comparatively higher than other altitudes at 800-1000 m, 1400-1600 m altitude in ripened and mature green stage.

In C- N stretch (aliphatic amines) 1401 cm^{-1} (800-1000 m) altitude in mature green and ripened stage was recorded with the maximum frequency which was followed by 1216 cm^{-1} (1400-1600 m at ripened stage).

Likewise, C-H wag (alkyl halides) 1400-1600 m with 1175cm^{-1} and 1187 cm^{-1} (>1600 m altitude) was found.

Moreover, C-O stretch (alcohol) was recorded with 1185 cm^{-1} (s), 1073 cm^{-1}(s) at 800-1000 m, 1219 cm^{-1}(s), 1076 cm^{-1}(s) (1000-1200 m) and 1217 cm^{-1} (vs), 1112 cm^{-1} (vs) at 1400-1600 m altitude.

Further, rough lemon showed sharp and strong peak (O-H bond) at 3168 cm^{-1} (800-1000 m), 3407 cm^{-1} (1400-1600 m and >1600 m). C-H stretch in the frequency of 2932 cm^{-1}, 2957 cm^{-1}, 2928 cm^{-1}, 2917 cm^{-1}, 2917 cm^{-1} at 800-1000 m, 1000-1200 m, 1200-1400 m .1400-1600 m and >1600 m.

Further C=O bond was found at 1717 cm^{-1}, 1724 cm^{-1}, 1734 cm^{-1} at 800-1000 m, 1000-1200 m and 1200-1400 m. Likewise, C-O bond at (1210, 1072) cm^{-1} at 800-1000 m, (1210, 1198, 1072) cm^{-1} at 1000-1200 m.

In addition, C-N stretch (1018 cm^{-1}) at 800-1000 m; 1225 cm^{-1}, 1067 cm^{-1}, 1043 cm^{-1} at 1200-1400 m; 1217 cm^{-1} ,1019 cm^{-1}(1400-1600 m, >1600 m). Further C=C bond was found at 1600 cm^{-1} (800-1000 m), 1696 cm^{-1} (1200-1400 m); 1717 cm^{-1}, 1617 cm^{-1} (1400-1600 m and >1600 m).

Table No. 4.4.4: Functional groups present in pectin at different altitudinal range and two maturity stages

Functional group	Bond	Germplasm	Frequency (cm^{-1})									
			800-1000 m		1000-1200 m		1200-1400 m		1400-1600 m		>1600 m	
			Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Alcohol, phenol	O-H	Sikkim mandarin	3364(s,sh)	3607(s,sh)	-	3135(s,sh)	3585(s,sh)	3537(s,sh)	-	-	3604 (s,sh)	-
		Pommelo	3575(s, sh)	3618, 3012(s,sh)	-	3503(s,sh)	3596,3046 (s,sh)	3173(s,sh)	-	3034(s,sh)	3552 (s,sh)	3349(s,sh)
		Rough lemon	-	3168(s,sh)	3569 (s,sh)	-	3401(s,sh)	-	-	3407(s,sh)	-	3407(s,sh)
Alkane	C-H stretch	Sikkim mandarin	2949(s)	2956(s)	2921(s)	2939(s)	-	2957(s)	2952(s)	3048(s)	2957 (s)	2959(s)
		Pommelo	3057(s) 2937(s)	2938(s)	3058,2936 (s)	3098(s)	2943(s)	2935(s)	2921(s)	2952(s)	3098, 2955(s)	2953(s)
		Rough lemon	2954(s)	2932(s)	3062,2992 (s)	2957,3036 (s)	2950(s)	2928(s)	1191(s)	2917(s)	3058 2959(s)	2917(s)
	C-H wag ($-CH_2X$)	Sikkim mandarin	-	-	-	-	-	-	-	1103(m)	-	1108 (m)
		Pommelo	-	-	-	-	-	-	-	1175(m)	-	1187(m)
		Rough lemon	-	-	-	-	1191 (m)	-	1011 (m)	-	-	-
α, B unsaturated ester	C=O stretch	Sikkim mandarin	1727(s)	1727(s)	1718(s)	1721(s)	1714(s)	1721(s)	1717(s)	1712(s)	1696(s)	1715(s)
		Pommelo	1723(s) 1689(s)	1718(s)		1718(s)	-	1720(s)	-	1723,1610 (s)	-	1719(s)
		Rough lemon	1710(s)	1717(s)	1717(s)	1724(s)	1694(s)	1734(s)	1723(s)		1716(s)	-
Alcohols, carboxylic	C-O stretch	Sikkim mandarin	-	1204 (v,s)	1109	-	-	-	-	-	1210 (v,s)	-

acids, esters, ethers		Pommelo	1202(v,s)	1185,1073	-	1219, 1076(v,s)	-	1217,1112 (v,s)	-	-	-	-
		Rough lemon	-	1210,1072 (v,s)	-	1210,1198, 1072(v,s)	-	-	-	-	-	-
Aliphatic amines	C-N stretch	Sikkim mandarin	1026(m)	1075,1201(m)	1222,1076 (m)	1073,1219 (m)	1229, 1075 1018(m)	1074, 1214 (m)	1990,1339 , (s) 1206,1074 (m)	1017,1064 (m)	1075 ,10 19(m)	1021,1095 (m)
		Pommelo	1401(s) 1061(m)	1401(s),1061 (m)	1069(m)	1074,1001 (m)	1071,1009 (m)	1212,1006 (m)	1098,1023 (m)	1216,1020 (m)	-	1076(m)
		Rough lemon	1072 (m)	1018(m)	1223,1074 , 1020(m)	-	-	1225,1067, 1043(m)	1403,1219 (m)	1217(m)	1074(m)	1019(m)
Alkyl halides	C-Cl stretch	Sikkim mandarin	-	-	-	-	-	-	-	883(m)	-	-
		Pommelo	-	-	-	856(m)	825(m)	839(m)	-	833(m)	-	-
		Rough lemon	-	-	-		-	-	-	-	-	-
Primary amides	N-H bend	Sikkim mandarin		-	-		-	-	-	-	-	-
		Pommelo	-	-	-	-	-	-	1621(w,m)	-	-	-
		Rough lemon	-	-	-	-	-	-		-	-	-

4.7. Ionome profiling of peel and pomace of Citrus germplasm

The ionome profiling of citrus germplasm in peel and pomace had been done to analyze the quantity of elements *viz*: major, minor (trace) and heavy elements present in it.

4.7.1. Essential elements in peel

The peel of citrus germplasm was subjected to essential element analysis which are illustrated in Table 4.7.1-4.7.9 and explained in the following sub heads:

4.7.1.1 Nitrogen (ppm)

Three factor analysis of variance is shown in Table No. 4.7.1. Sikkim mandarin was found to be superior having highest peel nitrogen up to 5016.67 ppm at an altitude range of 1400-1600 m at ripened stage which was found to be at par with 4976.67 ppm (800-1000 m altitude) in ripened stage. Whereas the lowest nitrogen content was 3123.33 ppm (1400-1600 m altitude) in mature green stage.

In Pommelo, peel nitrogen was recorded up to 4900 ppm (1400-1600 m altitude), which was followed by 4623 ppm (1400-1600 m altitude) in ripened stage. Whereas the lowest was 3333.33 ppm (800-1000 m altitude) in ripened stage.

Whereas in rough lemon it was 5000 ppm nitrogen at 1200-1400 m altitude in ripened stage as the highest and 3300 ppm (>1600 m altitude) as the least in mature green stage.

The lowest peel nitrogen content during the experiment was observed in Sikkim mandarin (3,233.33 ppm) at 1200-1400 m altitude in mature green stage.

There is no uniformity of increase in nitrogen content with regards to increase in altitude.

In all the cases, ripened stage of the fruit was recorded with higher peel moisture than the mature green stage.

4.7.1.2 Phosphorus (P) ppm

The data regarding peel phosphorus as shown in Table No 4.5.1 revealed that the Sikkim mandarin at ripened stage collected from 800-1000 m altitude had highest value of 245 ppm amongst all the interactions. It was found to be at par with 235 ppm (>1600 m) altitude in pommelo, 239.33 ppm (800-1000 m altitude) in rough lemon at ripened stage, 236.67 ppm (1000-1200 m altitude) in ripened stage and 235.67 ppm (1200-1400 m altitude) in ripened stage.

Whereas the least value (235 ppm) was recorded for pommelo at ripened stage collected from (>1600 m altitude) which was at par with 231.67 ppm (>1600 m altitude) in mature green stage. Whereas the least phosphorus content was recorded at 800-1000 m altitude (125 ppm) in mature green stage.

In regards to rough lemon, 239.33 ppm (800-1000 m altitude) showed the highest phosphorus content which was at par with 236.67 ppm (1000-1200 m altitude) in ripened stage and 235.67 ppm (1200-1400 m altitude) in ripened stage.

There was increasing trend in peel phosphorus content with increased altitude in case of pommelo. However, no such trend was found for rough lemon and Sikkim mandarin.

Moreover, ripened fruit shows much higher peel phosphorus content as compared to mature green stage irrespective of the different germplasm and stages.

4.7.1.3 Potassium (K) ppm

The potassium content in peel was highest in Sikkim mandarin collected from > 1600 m (41.10 %) at ripened stage which is followed by rough lemon and pommelo.

Pommelo was found with 2250 ppm (>1600 m altitude) in ripened stage as the highest which was at par with 2156 ppm (1200-1400 m altitude) in ripened stage.

On the other hand, rough lemon showed 2266.67 ppm (>1600 m altitude) in ripened stage as the maximum potassium content which was at par with 2200 ppm (1400-1600 m altitude) in ripened stage and 2,216.67 (>1600 m altitude) in mature green stage.

There is increasing potassium content with increasing altitude in pommelo. However, no such trend was observed for Sikkim mandarin and rough lemon.

Further ripened stage was found with higher potassium content than the mature green stage with respect to different germplasm and stages (Tble 4.5.1).

Table No 4.5.1: Nitrogen, phosphorus and potassium content (ppm) in peel of citrus germplasms at different altitude and different maturity stages

Germplasm	Altitude (m)	Nitrogen		Phosphorus		Potassium	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	4,242.11	4,976.67	145.00	245.00	1,050.00	1,970.96
	1000-1200	3,625.00	4,056.67	193.33	223.78	1,521.00	1,826.67
	1200-1400	3,233.33	4,333.33	154.67	216.67	1,093.22	2,216.67
	1400-1600	3,123.33	5,016.67	196.22	221.67	1,343.00	2,150.00
	>1600	4,158.78	4,442.33	175.00	215.00	1,643.14	2,600.00
Pommelo	**800-1000**	3,333.33	3,666.67	125.00	150.00	1,100.00	1,340.00
	1000-1200	3,564.40	4,400.00	175.00	180.00	1,500.00	2,050.00
	1200-1400	3,561.00	4,341.33	186.67	188.33	1,966.67	2,073.33
	1400-1600	4,623.00	4,900.00	192.33	210.00	2,033.33	2,156.00
	>1600	3,611.13	4,200.00	231.67	235.00	2,123.33	2,250.00
Rough lemon	**800-1000**	3,475.56	3,519.00	117.00	239.33	1,366.68	1,933.33
	1000-1200	3,566.67	3,700.00	217.00	236.67	1,756.67	1,940.00
	1200-1400	3,575.52	5,000.00	187.00	235.67	1,406.67	1,684.67
	1400-1600	4,368.00	4,580.00	129.67	188.00	1,288.33	2,200.00
	>1600	3,300.00	3,833.33	181.67	205.33	2,216.67	2,266.67

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	**54.02**	**26.99**	**19.09**	**3.48**	**1.74**	**1.23**	**42.56**	**21.27**	**15.04**
Factor(A)	**41.84**	**20.91**	**14.79**	**4.49**	**2.25**	**1.59**	**54.94**	**27.46**	**19.42**
Factor(S)	**34.16**	**17.07**	**12.07**	**2.84**	**1.42**	**1.01**	**34.75**	**17.37**	**12.28**
Interaction (GXAXS)	**132.32**	**66.13**	**46.76**	**11.01**	**5.50**	**3.89**	**134.57**	**67.26**	**47.56**

4.7.1.4 Calcium (ppm)

The highest calcium content was recorded in peel of ripened rough lemon followed by Sikkim mandarin and pommelo (Table 4.5.2)

Rough lemon grown at 800-1000 m altitude was recorded with 287.81 ppm calcium content in ripened stage. Whereas the lowest was 123.69 ppm (1200-1400 m altitude) in mature green stage for rough lemon.

However, in ripened stage, pommelo was recorded with 163.33 ppm (1400-1600 m altitude) as the highest calcium content. It was found to be statistically at par with 158.43 ppm (1000-1200 m altitude) in ripened stage.

Furthermore, Sikkim mandarin was observed with 180.49 ppm (1400-1600 m altitude) as the maximum content in calcium in ripened stage and the lowest record was found in 800-1000 m i.e. 147.84 ppm in mature green stage.

The increase in peel calcium content was not in uniformity with increasing altitude for all the germplasm. Moreover, ripened fruit shows much higher peel calcium content as compared to mature green stage irrespective of the different germplasm and stages.

4.7.1.5 Magnesium (ppm)

The highest magnesium content was recorded in peel of mature green rough lemon followed by Sikkim mandarin and pommelo (Table 4.5.2).

Rough lemon grown at 800-1000 m altitude was recorded with 422.33 ppm magnesium content.

It was followed by 419.33 ppm (00-1000 m altitude) in ripened stage as shown in Table 4.3.20. On the other hand, pommelo was found with 444.00 (>1600 m altitude) in ripened stage as the maximum content in magnesium and was followed by 416.98 ppm (>1600 m altitude) in mature green stage.

In Sikkim mandarin highest magnesium content was 422.33 ppm at >1600 m altitude in the mature green stage which was in turn followed by 421.33 ppm (1000-1200 m altitude) in mature green stage. The increase in peel magnesium content was not in uniformity with increasing altitude. Likewise, peel magnesium content was also found much higher for mature green fruit than ripened stage irrespective of germplasm and altitude.

4.7.1.6 Sulphur (ppm)

The peel of ripened samples of rough lemon collected from 800-1000 m altitude accounted for highest sulphur (24.79 ppm) which was followed by 19.18 ppm (1200-1400 m altitude) in ripened stage. On the other hand, highest sulphur content in pommelo was to the tune of 21.68 ppm at ripened stage which was at par with 20.20 ppm (1400-1600 m altitude) in ripened stage (Table 4.5.2)

In addition, Sikkim mandarin showed 20.67 ppm (800-1000 m altitude) as the highest which was found to be at par with 20.40 ppm (1400-1600 m altitude) in ripened stage.

Whereas the lowest was 10.38 ppm (800-1000 m altitude) in mature green stage for rough lemon out of all 30 combination.

It was also noticed that in pommelo there was increasing trend in sulphur content with increasing altitude. However, no such trend was observed for Sikkim mandarin and rough lemon. The ripened stage also showed much higher peel sulphur content as compared to mature green stage irrespective of all the germplasm and two stages.

Table No. 4.5.2: Calcium, magnesium and sulphur content (ppm) in peel of citrus germplasm at five different altitude and different maturity stages

Germplasm	Altitude (m)	Calcium		Magnesium		Sulphur	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	147.84	157.20	421.00	403.33	10.45	20.67
	1000-1200	149.31	169.68	421.33	374.65	13.33	18.85
	1200-1400	154.46	157.79	420.33	418.67	14.37	18.97
	1400-1600	156.62	180.49	418.00	393.33	14.15	20.40
	>1600	156.23	162.06	422.33	415.67	13.35	17.36
Pommelo	**800-1000**	123.29	153.50	277.33	355.33	13.33	14.28
	1000-1200	133.00	158.43	276.67	334.00	13.48	16.53
	1200-1400	131.79	156.00	321.71	401.33	15.33	18.63
	1400-1600	155.30	163.33	355.52	312.25	15.63	20.20
	>1600	142.52	150.67	416.98	418.00	15.94	21.68
Rough lemon	**800-1000**	245.67	287.81	422.33	415.67	10.38	24.79
	1000-1200	247.82	258.74	408.00	413.67	14.87	16.18
	1200-1400	123.69	264.66	407.33	407.67	19.11	19.18
	1400-1600	167.00	255.33	419.33	413.00	13.04	18.38
	>1600	182.66	247.16	416.33	414.33	14.16	15.55

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	**1.66**	**0.83**	**0.58**	**2.81**	**1.41**	**0.99**	**0.83**	**0.41**	**0.29**
Factor(A)	**2.14**	**1.07**	**0.76**	**3.63**	**1.82**	**1.28**	**1.07**	**0.53**	**0.37**
Factor(S)	**1.36**	**0.68**	**0.48**	**2.30**	**1.15**	**0.81**	**0.68**	**0.33**	**0.24**
Interaction (GXAXS)	**5.25**	**2.63**	**1.86**	**8.90**	**4.45**	**3.14**	**2.62**	**1.29**	**0.91**

4.7.1.7 Iron (ppm)

The iron content in peel was highest in Sikkim mandarin collected from 800-1000 m altitude (3.13 ppm) at ripened stage which is statistically at par with 2.98 ppm in Sikkim mandarin collected from 1200-1400 m altitude in ripened stage. In mature green stage, highest peel iron (2.68 ppm) was estimated in the Sikkim mandarin samples collected from 800-1000 m and lowest was in the samples of rough lemon (1.10 ppm) at 1400-1600 m altitude. (Table 4.5.3)

In addition, pommelo collected from >1600 m altitude in ripened stage was found with highest iron content (2.11 ppm) and that of 1.40 ppm (1200-1400 m altitude) in mature green stage showed the maximum content of iron. On the other hand, rough lemon was found with 2.10 ppm (800-1000 m altitude) in ripened stage as the highest iron content and 1.63 ppm (>1600 m altitude) in mature green stage was recorded the maximum iron content.

There was no pattern of increase in iron content with respect to increasing altitude in all the germplasms. Peel boron content was found to be highest for ripened fruit than the mature green stage irrespective of the germplasms and altitude.

4.7.1.8 Manganese (ppm)

The higher peel manganese content was observed in pommelo followed by rough lemon and Sikkim mandarin. The highest manganese content was recorded in the ripened samples collected from >1600 m altitude (0.14 ppm) in pommelo. It was found to be at par with 0.11 ppm (1400-1600 m altitude) in ripened stage, 0.10 ppm (1200-1400 m altitude) in ripened stage, 0.10 ppm (>1600 m altitude) in mature green stage. Whereas the least was 0.03 ppm (800-1000 m altitude) in mature green stage (Table 4.5.3)

In addition, Sikkim mandarin resulted to 0.05 ppm (800-1000 m altitude) in ripened stage as the highest and that of 0.01 ppm (1200-1400 m altitude) in mature green stage as the lowest manganese content.

On the other hand, rough lemon was found with 0.08 ppm (>1600 m altitude) in ripened stage as the highest and 0.02 ppm at 1000-1200 m, 1200-1400 m, 1400-1600 m altitude in mature green stage and 1400-1600 m altitude in ripened stage as the lowest manganese content.

In regard to altitude, there was trend of increase with increasing altitude for pommelo. However, no such uniformity with respect to increase in altitude was found for Sikkim mandarin and rough lemon.

Moreover, peel manganese content was found to be higher in ripened stage than mature green irrespective of germplasms and altitude.

4.7.1.9 Copper (ppm)

The data regarding peel ash as shown in Table No 4.5.3 revealed that the Sikkim mandarin at ripened stage collected from 1200-1400 m altitude had highest value of 18.03 ppm copper content amongst all the interactions. Whereas the least value was recorded for pommelo collected at mature green stage from 1400-1600 m altitude (12.92 ppm).

On the other hand, pommelo peel was recorded with 18.00 ppm at 1200-1400 m altitude in ripened stage as the highest and 14.00 ppm (1000-1200 m in ripened stage) and 1400-1600 m altitude in mature green stage showed the lowest copper content.

Likewise, rough lemon was recorded with 16.29 ppm (1000-1200 m altitude) in ripened stage as the highest and 12.23 ppm (>1600 m altitude) at mature green stage as the lowest. There was no increasing trend with respect to increase in altitude for all the germplasm.

Moreover, ripened fruit shows much higher peel copper content as compared to mature green stage irrespective of the different germplasm and stages.

Table No. 4.5.3: Iron, manganese and copper content (ppm) in peel of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Iron		Manganese		Copper	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	2.68	3.13	0.04	0.05	16.21	15.54
	1000-1200	1.48	1.52	0.03	0.04	14.39	17.67
	1200-1400	2.02	2.98	0.01	0.04	17.44	18.03
	1400-1600	1.28	2.44	0.02	0.04	12.92	14.53
	>1600	1.79	2.56	0.03	0.03	16.00	17.00
Pommelo	**800-1000**	1.53	1.60	0.03	0.05	16.67	15.92
	1000-1200	1.50	1.73	0.04	0.09	16.33	14.00
	1200-1400	1.40	1.71	0.06	0.10	16.67	18.00
	1400-1600	1.52	1.90	0.06	0.11	14.00	14.67
	>1600	1.85	2.11	0.10	0.14	17.00	14.33
Rough lemon	**800-1000**	1.30	2.10	0.03	0.04	13.96	12.67
	1000-1200	1.23	1.87	0.02	0.04	13.10	16.29
	1200-1400	1.40	1.43	0.02	0.03	12.70	14.00
	1400-1600	1.10	1.70	0.02	0.02	12.68	15.33
	>1600	1.63	1.77	0.05	0.08	12.23	12.33

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	**0.09**	**0.05**	**0.03**	**0.010**	**0.007**	**0.005**	**0.59**	**0.29**	**0.21**
Factor(A)	**0.12**	**0.06**	**0.04**	**0.020**	**0.009**	**0.006**	**0.76**	**0.38**	**0.27**
Factor(S)	**0.08**	**0.04**	**0.03**	**0.010**	**0.006**	**0.004**	**0.48**	**0.24**	**0.17**
Interaction (GXAXS)	**0.29**	**0.15**	**0.10**	**0.040**	**0.02**	**0.016**	**1.85**	**0.93**	**0.65**

4.7.1.10 Molybdenum (ppm)

The three-way interactions of germplasms, altitude range and the stage of maturity (Table 4.5.4), depicts the presence of highest molybdenum content (1.70 ppm) in ripened stage of pommelo grown at at 1200-1400 m altitude as compared to all other interactions. It was followed by 1.17 ppm (1000-1200 m altitude) in the ripened stage. Likewise, Sikkim mandarin showed the highest molybdenum content to the tune of 0.75 ppm in the samples collected from >1600 m altitude in the ripened stage.

In addition, rough lemon showed (0.77 ppm) at 1000-1200 m altitude in ripened stage as the maximum value which was at par with 0.73 ppm (1200-1400 m altitude) in ripened stage.

As such there was no such trend of increase in peel molybdenum content with increase in altitude for all the germplasm. The ripened stage also showed much higher mercury content as compared to mature green stage irrespective of all the germplasm and two maturity stages.

4.7.1.12 Boron (ppm)

The highest boron content was 2.86 ppm in peel of ripened rough lemon fruits collected from >1600 m altitude which was at par with 2.62 ppm (1000-1200 m altitude) in the ripened stage and 2.57 ppm (1400-1600 m altitude) in the ripened stage. The lowest content was recorded in pommelo (1.02 ppm) of mature green fruit from 1200-1400 m altitude (Table 4.5.4).

In Pommelo peel boron was highest i.e. 2.08 ppm at 1400-1600 m altitude at ripened stage which was followed by 2.02 ppm (>1600 m altitude) in the ripened stage.

On the other hand, Sikkim mandarin was recorded with 2.48 ppm (1200-1400 m altitude) in the ripened stage which was at par with 2.47 ppm (1400-1600 m altitude) in the ripened stage.

The increase in boron peel was irrespective of increase in altitude in all the germplasms. Further, boron peel was much higher for ripened fruit than mature green stage for all the germplasms.

4.7.1.10 Zinc (ppm)

Rough lemon showed the highest zinc content followed by pommelo and Sikkim mandarin. Further rough lemon 0.87 ppm (800-1000 m altitude) in the ripened stage showed the highest. In addition, pommelo (0.81 ppm) at 1400-1600 m altitude in the ripened stage was recorded with the highest zinc content. It was followed by 0.67 ppm (1400-1600 m altitude) in the ripened stage as the next highest in pommelo.

On the other hand, Sikkim mandarin showed 0.70 ppm (1200-1400 m) altitude in the ripened stage as the highest zinc content. It was found to be at par with 0.66 ppm (800-1000 m) altitude in the ripened stage as well as 0.68 ppm (800-1000 m), 0.67 ppm (1000-1200 m) altitude in the ripened stage.

Moreover, rough lemon was found with 0.87 ppm (800-1000 m altitude) in the ripened stage as the highest zinc content. It was followed by 0.70 ppm (1000-1200 m altitude) in the ripened stage (Table 4.5.4).

Table No 4.5.4: Molybdenum, boron, zinc content (ppm) in peel of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude(m)	Molybdenum		Boron		Zinc	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	0.37	0.52	1.91	2.01	0.66	0.68
	1000-1200	0.47	0.39	1.87	2.09	0.26	0.67
	1200-1400	0.39	0.47	1.59	2.48	0.53	0.70
	1400-1600	0.38	0.66	1.20	2.47	0.26	0.40
	>1600	0.62	0.75	1.62	1.63	0.30	0.43
Pommelo	**800-1000**	0.51	0.91	1.09	1.10	0.46	0.50
	1000-1200	0.64	1.17	1.09	1.37	0.43	0.43
	1200-1400	0.67	1.70	1.02	1.52	0.54	0.57
	1400-1600	0.80	1.04	1.53	2.08	0.67	0.91
	>1600	0.73	1.13	1.25	2.02	0.48	0.61
Rough lemon	**800-1000**	0.43	0.63	1.53	1.94	0.53	0.87
	1000-1200	0.40	0.77	1.69	2.62	0.51	0.70
	1200-1400	0.64	0.73	2.08	2.50	0.50	0.52
	1400-1600	0.38	0.44	1.47	2.57	0.32	0.40
	>1600	0.27	0.42	2.53	2.86	0.37	0.55

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	**0.04**	**0.02**	**0.02**	**0.10**	**0.05**	**0.04**	**0.02**	**0.01**	**0.01**
Factor(A)	**0.06**	**0.03**	**0.02**	**0.13**	**0.06**	**0.05**	**0.03**	**0.02**	**0.01**
Factor(S)	**0.04**	**0.02**	**0.01**	**0.08**	**0.04**	**0.03**	**0.02**	**0.01**	**0.01**
Interaction (GXAXS)	**0.14**	**0.07**	**0.05**	**0.31**	**0.16**	**0.11**	**0.08**	**0.04**	**0.03**

4.7.2 Trace elements in citrus peel

4.7.2.1 Aluminum (ppm)

Three factor analysis of variance was shown in Table No. 4.5.5. Sikkim mandarin was found to be superior having highest aluminum content up to 1.59 ppm at an altitude range of 800-1000 m altitude at ripened stage which was found to be at par with 1.50 ppm (1200-1400 m altitude) , 1.37 ppm (>1600 m) altitude and 1.30 ppm (1400-1600 m altitude) in mature green stage.

In Pommelo, aluminum content was recorded up to 1.57 ppm (1200-1400 m altitude) in ripened stage as the maximum value. It was in turn at par with 1.44 ppm (1200-1400 m altitude), 1.47 ppm (1400-1600 m altitude) , 1.37 ppm (800-1000 m altitude) and 1.20 ppm (>1600 m altitude) in the mature green stage as well as 1.33 ppm (1000-1200 m altitude), 1.40 ppm (>1600 m altitude) in the ripened stage.

Whereas in rough lemon it was 1.39 ppm (800-1000 m altitude) in the ripened stage as the maximum value.

There was no increasing pattern of aluminium with response to increase in altitude for all the germplasm. Ripened stage was higher than mature green stage irrespective of germplasm and stages.

4.7.2.3 Cobalt (ppm)

The cobalt content of fruit peel was found to be highest (0.23 ppm) at the ripened stage of pommelo grown at an altitude of >1600 m altitude compared to other germplasms grown at different altitudes which was found to be at par with 0.19 ppm (1000-1200 m and 1200-1400 m altitude) in the ripened stage (Table 4.5.5)

On the other hand, Sikkim mandarin had the highest cobalt content to the tune 0.19 ppm at (1400-1600 m altitude) at ripened stage which was in turn at par with 0.18 ppm (>1600 m altitude) and 0.14 ppm (1000-1200 m altitude) in the ripened stage. Further it was also at par with 0.17 ppm, 0.15 ppm at mature green stage in 1400-1600 m altitude and >1600 m altitude. The lowest cobalt content was 0.12 ppm at ripened stage rough lemon collected from 800-1000 m altitude. It was found to be statistically at par with 0.11 ppm (1000-1200 m), 0.10 ppm (1200-1400 m altitude), 0.09 ppm (1400-1600 m altitude) , 0.07 ppm (>1600 m altitude) altitude in the ripened stage as well as in mature green stage with 0.10 ppm (800-1000 m altitude), 0.09 ppm (1200-1400 m altitude) and 0.07 ppm (1400-1600 m altitude).

The ripened peel was found to have much higher cobalt content than the mature green stage irrespective of germplasm and stages.

The increase in peel cobalt content was not in uniformity with increasing altitude for all the germplasm.

4.7.2.2. Sodium (ppm)

The highest sodium content was recorded in peel of ripened rough lemon and Sikkim mandarin followed by pommelo. Sikkim mandarin and rough lemon grown at 1400-1600 m and 1000-1200 m altitude was recorded with 27.33 ppm sodium content as shown in Table 4.5.5.

Furthermore, pommelo was recorded with 23.42 ppm (800-1000 m altitude) in the ripened stage as the highest sodium content and 13.67 ppm (800-1000 m altitude) in the mature green stage as the lowest sodium content.

In rough lemon (27.33 ppm) at 1000-1200 m altitude in the ripened stage was found with the highest sodium content and that of the lowest (15.54 ppm) in 1200-1400 m altitude in the mature green stage.

Furthermore, Sikkim mandarin was recorded with 27.33 ppm (1200-1400 m altitude) as the maximum in ripened stage and that of 15.48 ppm (800-1000 m altitude) in the mature green stage as the lowest sodium content.

The increase in peel sodium content was not in uniformity with increasing altitude. Likewise peel sodium content was also found much higher for ripened fruit than the mature green stage irrespective of altitude and germplasm.

Table No 4.5.5: Aluminium, cobalt, sodium content (ppm) in peel of citrus germplasm at different altitude and differentmaturity stages

Germplasm	Altitude(m)	Aluminium		Cobalt		Sodium	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	1.15	1.59	0.05	0.10	15.48	16.15
	1000-1200	1.03	1.11	0.08	0.14	16.67	19.73
	1200-1400	1.50	1.10	0.05	0.10	18.29	22.06
	1400-1600	1.30	1.13	0.17	0.19	16.79	27.33
	>1600	1.37	0.87	0.15	0.18	16.29	16.60
Pommelo	800-1000	1.37	1.03	0.11	0.15	13.67	23.42
	1000-1200	1.03	1.33	0.12	0.19	14.48	16.67
	1200-1400	1.44	1.57	0.12	0.19	16.33	17.00
	1400-1600	1.47	1.10	0.13	0.15	16.07	21.33
	>1600	1.20	1.40	0.10	0.23	14.00	18.00
Rough lemon	800-1000	1.27	1.39	0.10	0.12	16.70	17.36
	1000-1200	1.17	1.35	0.05	0.11	18.67	27.33
	1200-1400	1.23	1.33	0.09	0.10	15.54	22.82
	1400-1600	1.18	1.28	0.07	0.09	16.50	23.22
	>1600	1.23	1.34	0.05	0.07	18.17	26.33

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	0.12	0.06	0.04	0.02	0.01	0.01	0.58	0.29	0.21
Factor(A)	0.16	0.08	0.06	0.02	0.01	0.01	0.76	0.38	0.27
Factor(S)	0.10	0.05	0.04	0.01	0.01	0.01	0.48	0.24	0.17
Interaction (GXAXS)	0.38	0.19	0.14	0.05	0.03	0.02	1.85	0.93	0.65

4.7.2.3 Lithium (ppm)

The highest peel lithium content was recorded in pommelo followed by rough lemon and Sikkim mandarin. There was increasing trend with altitude for Sikkim mandarin both at ripened and mature green stages. However, no such trend was observed for rough lemon and pommelo. The peel lithium content was also found to be much higher for ripened fruit than the mature green stage irrespective of germplasm and altitude (Table 4.5.6).

In pommelo peel, lithium content was highest i.e. 1.08 ppm at >1600 m altitude at ripened stage which was followed by 0.76 ppm (1400-1600 m altitude) in the ripened stage. Likewise, in rough lemon (0.76 ppm) at 1400-1600 m altitude at ripened stage showed the highest lithium content which was found to be at par with 0.73 ppm (>1600 m altitude) and 0.70 ppm (1000-1200 m altitude) in ripened stage.

On the other hand, Sikkim mandarin was recorded with 0.43 ppm (>1600 m altitude) in ripened stage as the highest value. It was found to be at par with 0.39 ppm (1400-1600 m altitude) in ripened and mature green stage as well as 0.40 ppm (>1600 m altitude) in mature green stage.

4.7.2.4 Barium (ppm)

The peel moisture as revealed from the three-way interaction of germplasm x altitude x stage of maturity (Table No. 4.5.6). Peel of rough lemon was observed to have 34.48 ppm at 1000-1200 m altitude at ripened stage which is statistically at par with 32.67 ppm (800-1000 m altitude), 34.43 ppm (1200-1400 m altitude) and 32.33 ppm (>1600 m altitude) in ripened stage.

Highest barium content (32.33 ppm) in pommelo was recorded in the samples collected from 1400-1600 m altitude at ripened stage which was at par with 31.60 ppm (1200-1400 m altitude) in ripened stage and 30 ppm (1400-1600 m altitude) in mature green stage.

In Sikkim mandarin, the highest value was seen to be 30 ppm in the ripened samples collected from (1400-1600 m altitude) which was followed by 25 ppm in >1600 m altitude in ripened stage.

There was no pattern of increase in peel barium with respect to increasing altitude in all the germplasms. Peel barium content was found to be highest for ripened fruit than the mature green stage irrespective of the germplasms and altitude.

4.7.2.5 Nickel (ppm)

The peel nickel content as revealed from the three-way interaction of germplasm X altitude X stage of maturity is illustrated in Table No. 4.5.6. Peel of Sikkim mandarin was observed to have 0.69 ppm at 1000-1200 m altitude, 1400-1600 m altitude in the ripened stage as the highest nickel content. It was at par with 0.57 ppm (1200-1400 m altitude), 0.65 ppm (>1600 m altitude) in ripened stage and 0.55 ppm (1400-1600 m altitude), 0.54 ppm (>1600 m altitude) in the mature green stages.

Further pommelo also was observed with 0.69 ppm as highest in 1200-1400 m altitude in the ripened stage which was followed by 0.50 ppm (>1600 m altitude) in the ripened stage.

On the other hand rough lemon was observed with 0.66 ppm (>1600 m altitude) in the ripened stage as the maximum nickel content which was at par with

0.51 ppm at 1000-1200 m, >1600 m in the mature green stage and in ripened stage with 0.56 ppm (1200-1400 m), 0.53 ppm (1400-1600 m altitude).

Whereas, the lowest was recorded for ripened stage pommelo (0.15 ppm) from 1200-1400 m altitude in the ripened stage. There is no pattern of increase in nickel content with regards to increasing altitudes for all the germplasm. Moreover, the comparison among stages showed ripened stage with much higher nickel contain than the mature green stage irrespective of the germplasm and stages.

Table No 4.5.6: Lithium, barium, nickel content (ppm) in peel of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Lithium		Barium		Nickel	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	0.13	0.20	16.33	23.33	0.25	0.38
	1000-1200	0.17	0.29	15.34	19.67	0.41	0.69
	1200-1400	0.28	0.29	19.42	21.46	0.23	0.57
	1400-1600	0.39	0.39	22.71	30.00	0.55	0.69
	>1600	0.40	0.43	16.18	25.00	0.54	0.65
Pommelo	**800-1000**	0.31	0.44	25.00	26.67	0.19	0.21
	1000-1200	0.30	0.68	22.00	25.33	0.16	0.46
	1200-1400	0.36	0.72	22.12	31.60	0.15	0.69
	1400-1600	0.73	0.76	30.00	32.33	0.19	0.50
	>1600	0.59	1.08	25.00	25.92	0.17	0.50
Rough lemon	**800-1000**	0.37	0.42	28.96	32.67	0.35	0.42
	1000-1200	0.51	0.70	27.00	34.48	0.51	0.37
	1200-1400	0.42	0.54	27.33	34.43	0.46	0.56
	1400-1600	0.60	0.76	25.33	31.00	0.41	0.53
	>1600	0.63	0.73	26.67	32.33	0.51	0.66

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	**0.03**	**0.01**	**0.01**	**1.05**	**0.77**	**0.55**	**0.05**	**0.03**	**0.02**
Factor(A)	**0.04**	**0.02**	**0.01**	**1.36**	**0.60**	**0.42**	**0.07**	**0.03**	**0.02**
Factor(S)	**0.02**	**0.01**	**0.01**	**0.86**	**0.49**	**0.35**	**0.04**	**0.02**	**0.02**
Interaction (GXAXS)	**0.09**	**0.04**	**0.03**	**3.33**	**1.89**	**1.34**	**0.16**	**0.08**	**0.06**

4.7.2.6 Silicon (ppm)

The highest silicon content in peel was 0.93 ppm in peel of ripened stage pommelo fruits collected from 1400-1600 m which was statistically at par with 0.87 ppm collected from 1200-1400 m altitude in ripened stage (Table 4.5.7)

Likewise, pommelo showed 0.31ppm (1000-1200 m altitude) in ripened stage as the maximum value. It was at par with 0.23 ppm at 800-1000 m altitude and >1600 m altitude in ripened stage; 0.29 ppm (1200-1400 m altitude) in ripened stage; 0.23 ppm (1200-1400 m altitude) in mature green stage and 0.26 ppm (1400-1600 m altitude) in ripened stage.

On the other hand, rough lemon showed 0.19 ppm (1200-1400 m) altitude in the ripened stage as the highest silicon content which has no significant different with silicon content in rest altitude in both stages.

The lowest silicon content was recorded in rough lemon to the tune of 0.12 ppm collected from 1200-1400 m altitude in mature green stage.

The increase in peel silicon content was irrespective of increase in altitude in all the germplasms except Sikkim mandarin which followed different trend. Further, peel silicon content was much higher for ripened fruit than mature green fruit for all the germplasms.

4.7.2.7 Iodine (ppm)

The iodine content of fruit peel was found to be highest (1.80 ppm) at the ripened stage of pommelo grown at an altitude of >1600 m altitude compared to other germplasms grown at different altitudes which was in turn found to be at par with 1.68

ppm (1400-1600 m altitude) in ripened stage as illustrated in Table 4.5.7. In addition, rough lemon had the highest peel iodine content to the tune of 1.56 ppm at >1600 m altitude which was found to be statistically at par with 1.48 ppm (1400-1600 m altitude) in ripened stage. On the other hand, ripened Sikkim mandarin (1.33 ppm) collected from 1400-1600 m altitude also showed the maximum iodine content which was found to be at par with 1.12 ppm (1000-1200 m altitude) in mature green stage, 1.20 ppm (1400-1600 m altitude) , 1.24 ppm in >1600 m altitude in ripened stage and also 1.17 ppm (1400-1600 m altitude) in the mature green stage.

Lowest peel iodine content was 0.57 ppm at mature green Sikkim mandarin collected from 800-1000 m altitude. The peel iodine content was comparatively higher for ripened fruit than that of mature green fruit irrespective of the altitude and stages. Furthermore, rough lemon showed increase in iodine content with increasing altitude. However, no such trend was found for Sikkim mandarin and pommelo germplasm.

4.7.2.8 Cerium (ppm)

From the ANOVA table it is clear that Ce content is significantly different ($p<0.05$) in all the main factors. It is also observed from the ANOVA table that all the 3-way interaction also found significant (Table 4.5.7).

The Ce content is significantly high in pommelo in its ripened stage at (>1600 m altitude) i.e. 0.22 ppm compared to all other 3-way combinations. On the other hand, rough lemon showed 0.18 ppm (1000-1200 m altitude) in ripened stage as the highest and was at par with 0.15 ppm (1200-1400 m, and >1600 m in ripened stage). Further it was also at par with ripened fruit at 1400-1600 m altitude (0.17 ppm).

The germplasm pommelo showed increase in cerium content with increase in altitude. However, no such trend was observed for Sikkim mandarin and rough lemon. Likewise, ripened stage was found to have much higher cerium content than mature green stage irrespective of altitude and germplasm.

Table No. 4.5.7. Silicon, iodine, cerium content (ppm) in peel of citrus germplasm at different altitude and different different maturity stages

Germplasm	Altitude(m)	Silicon		Iodine		Cerium	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	0.41	0.76	0.57	0.77	0.12	0.18
	1000-1200	0.44	0.82	0.97	1.12	0.11	0.15
	1200-1400	0.63	0.87	0.80	1.20	0.11	0.17
	1400-1600	0.64	0.93	1.17	1.33	0.06	0.15
	>1600	0.73	0.83	0.70	1.24	0.09	0.14
Pommelo	**800-1000**	0.17	0.23	1.03	1.40	0.11	0.11
	1000-1200	0.16	0.31	1.10	1.23	0.12	0.14
	1200-1400	0.23	0.29	1.13	1.26	0.14	0.20
	1400-1600	0.21	0.26	1.00	1.68	0.16	0.21
	>1600	0.19	0.23	1.20	1.80	0.17	0.22
Rough lemon	**800-1000**	0.13	0.18	1.07	1.10	0.09	0.13
	1000-1200	0.15	0.17	1.08	1.13	0.07	0.18
	1200-1400	0.12	0.19	1.17	1.25	0.13	0.15
	1400-1600	0.13	0.15	1.23	1.48	0.07	0.17
	>1600	0.15	0.17	1.33	1.56	0.10	0.15

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	0.03	0.01	0.01	0.07	0.03	0.02	0.01	0.01	0.01
Factor(A)	0.03	0.02	0.01	0.09	0.04	0.03	0.02	0.01	0.01
Factor(S)	0.02	0.01	0.01	0.06	0.03	0.02	0.01	0.01	0.004
Interaction (GXAXS)	0.08	0.04	0.03	0.22	0.11	0.08	0.04	0.02	0.02

4.7.2.9 Silver (ppm)

The highest silver content in peel was 0.86 ppm (800-1000 m altitude) in mature green Sikkim mandarin which was followed by 0.81 ppm (800-1000 m altitude) in ripened stage. While the lowest silver content was recorded in rough lemon with 0.09 ppm at 1400-1600 m altitude in ripened stage (Table 4.5.8).

Likewise, in pommelo (0.21 ppm) at 1000-1200 m altitude in ripened stage was found to be highest silver content. In addition, 0.11 ppm at 1400-1600 m altitude in ripened stage showed the least silver content.

Further, rough lemon was found with 0.19 ppm in the samples collected from >1600 m altitude at mature green stage as the maximum silver content and that of 0.09 ppm at 1400-1600 m altitude in ripend stage was found with the lowest silver content in rough lemon.

There is no increasing trend of silver content with increasing altitude in all the germplasm. Mature green stage was found to be higher silver content than the ripened stage in all the germplasm.

4.7.2.10 Caesium (ppm)

The highest caesium content was recorded in peel of ripened Sikkim mandarin followed by rough lemon and pommelo. Sikkim mandarin grown at >1600 m was recorded with 0.20 ppm caesium and at par value (0.17 ppm) in the sample collected at ripened stage from 1400-1600 m altitude as shown in Table 4.5.8.

Furthermore, pommelo was recorded with 0.05 ppm (>1600 m) altitude in ripened stage as the highest caesium content after Sikkim mandarin, at par values were recorded to be 0.02 ppm

(800-1000 m in ripened stage), 0.02 ppm in 1200-1400 m, 1400-1600 m altitude in the mature green stage. 0.03 ppm in 1000-1200 m, 1200-1400 m, 1400-1600 m altitude in ripened stage and also with 0.04 ppm (>1600 m altitude) in mature green stage.

In rough lemon highest caesium content was 0.18 ppm (1200-1400 m) altitude in ripened stage which was followed by 0.12 ppm (>1600 m altitude) in ripened stage.

The increase in peel caesium was not in uniformity with increasing altitude for Sikkim mandarin and rough lemon except for pommelo which showed increasing pattern of caesium with response to increase in altitude. Likewise, peel caesium was also found much higher for ripened fruit than the mature green stage irrespective of altitude and germplasm.

Table No. 4.5.8 Silver and caesium content (ppm) in peel of citrus germplasm at different altitude and different different maturity stages

Germplasm	Altitude(m)	Silver		Caesium	
		Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	0.86	0.81	0.02	0.03
	1000-1200	0.72	0.68	0.06	0.07
	1200-1400	0.80	0.72	0.06	0.12
	1400-1600	0.50	0.51	0.06	0.17
	>1600	0.54	0.40	0.05	0.20
Pommelo	800-1000	0.20	0.19	0.01	0.02
	1000-1200	0.21	0.18	0.01	0.03
	1200-1400	0.16	0.12	0.02	0.03
	1400-1600	0.14	0.11	0.02	0.03
	>1600	0.15	0.12	0.04	0.05
Rough lemon	800-1000	0.13	0.12	0.02	0.09
	1000-1200	0.11	0.11	0.04	0.07
	1200-1400	0.10	0.10	0.06	0.18
	1400-1600	0.11	0.09	0.04	0.10
	>1600	0.19	0.16	0.06	0.12

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	0.019	0.009	0.007	0.011	0.005	0.004
Factor(A)	0.025	0.012	0.009	0.014	0.007	0.005
Factor(S)	0.016	0.008	0.005	0.010	0.004	0.003
Interaction (GXAXS)	0.061	0.030	0.021	0.033	0.017	0.012

4.7.3 Heavy metals in citrus peels

4.7.3.1 Lead (ppm)

The highest lead content was 0.08 ppm in peel of ripened stage rough lemon fruits collected from >1600 m as well as pommelo (1000-1200 m altitude) in mature green stage (Table 4.5.9)

On the other hand, Sikkim mandarin showed 0.06 ppm (1400-1600 m altitude) as the highest.

Comparison of two stage of maturity do not show any trend. Rough lemon showed increase in lead content with respect to increase in altitude but Sikkim mandarin and pommelo do not follow such trend.

4.7.3.2 Cadmium (ppm)

The data regarding peel cadmium as shown in Table No 4.5.9 revealed that the pommelo collected from >1600 m altitude does not detect cadmium at mature green and ripened stage. Whereas the maximum value (0.04 ppm) was recorded for pommelo collected at mature green stage from 1400-1600 m altitude.

On the other hand, Sikkim mandarin peel was recorded with 0.04 ppm cadmium content at 800-1000 m altitude (ripened stage) and 1000-1200 m, >1600 m altitude in mature green stage as the highest content. Whereas the lowest cadmium content in peel was 0.01 ppm (1200-1400 m, 1400-1600 m altitude).

In rough lemon samples cadmium content of 0.04 ppm recorded as the highest in the ripened and mature green stage at >1600 m altitude and that of 1200-1400 m altitude was not detected with cadmium content both in mature green and ripened stage.

As such there is no trend with increasing altitude in all the germplasm with respect to increase in altitude. Moreover, ripened and mature green fruit was not with any proper trend.

4.7.3.3 Mercury (ppm)

The peel of ripened rough lemon grown at 1400-1600 m altitude was recorded with highest mercury content to the tune of 0.08 ppm. On the contrary, mercury content was not detected in 1000-1200 m altitude at ripened stage (Table No 4.5.9)

Whereas, the peel of ripened Pommelo fruit grown at 1400-1600 m altitude was recorded mercury content as 0.05 ppm as the maximum value. On the other hand, lowest mercury content was 0.02 ppm at 1000-1200 m (mature green stage) and 1200-1400 m altitude in ripened stage.

In Sikkim mandarin, 800-1000 m and 1200-1400 m altitude (ripened stage); 1000-1200 m (mature green stage) mercury content was not detected. Whereas the highest mercury content was 0.04 ppm at 1400-1600 m, >1600 m altitude in the mature green stage as well as 1000-1200 m altitude in ripened stage.

There was no such trend of increase in peel mercury content with increase in altitude for all the germplasm. The ripened stage also showed much higher mercury content as compared to mature green stage irrespective of all the germplasm and two stages.

4.7.3.4 Uranium (ppm)

The higher peel uranium was observed in Sikkim mandarin followed by pommelo and rough lemon. The highest uranium content was recorded in the ripened samples collected from 1400-1600 m (0.19 ppm) followed by ripened pommelo samples at >1600 m altitude (0.13 ppm) in the ripened stage. On the other hand, rough lemon showed 0.05 ppm (>1600 m altitude) in ripened stage as the highest uranium content. (Table 4.5.9).

There was increasing trend of uranium with increase in altitude for rough lemon. However, no such trend was found in Sikkim mandarin and pommelo. Comparison in the stages revealed ripened stage with much higher uranium content than the mature green stage irrespective of germplasm and stages.

Table No. 4.5.9: Lead, cadmium, mercury and uranium content (ppm) in peel of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Lead		Cadmium		Mercury		Uranium	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	0.01	0.02	0.03	0.04	0.01	0.00	0.01	0.04
	1000-1200	0.05	0.02	0.04	0.03	0.00	0.04	0.02	0.05
	1200-1400	0.03	0.03	0.01	0.02	0.02	0.00	0.01	0.04
	1400-1600	0.06	0.03	0.01	0.02	0.04	0.02	0.03	0.19
	>1600	0.04	0.02	0.04	0.03	0.04	0.03	0.04	0.17
Pommelo	800-1000	0.04	0.02	0.03	0.02	0.03	0.04	0.05	0.05
	1000-1200	0.08	0.02	0.01	0.03	0.02	0.05	0.03	0.12
	1200-1400	0.05	0.04	0.03	0.03	0.04	0.02	0.02	0.08
	1400-1600	0.00	0.02	0.04	0.02	0.04	0.04	0.02	0.10
	>1600	0.07	0.05	0.00	0.00	0.03	0.04	0.06	0.13
Rough lemon	800-1000	0.04	0.02	0.02	0.01	0.06	0.03	0.02	0.02
	1000-1200	0.06	0.03	0.02	0.03	0.06	0.00	0.02	0.02
	1200-1400	0.03	0.06	0.00	0.00	0.04	0.02	0.02	0.03
	1400-1600	0.04	0.07	0.03	0.02	0.04	0.08	0.01	0.03
	>1600	0.05	0.08	0.04	0.04	0.04	0.04	0.04	0.05

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	0.009	0.004	0.003	0.010	0.005	0.004	0.013	0.007	0.005	0.008	0.004	0.003
Factor(A)	0.011	0.006	0.004	0.012	0.007	0.005	0.016	0.008	0.006	0.011	0.005	0.004
Factor(S)	0.007	0.004	0.002	0.008	0.004	0.003	0.01	0.005	0.004	0.007	0.003	0.002
Interaction (GXAXS)	0.027	0.014	0.01	0.030	0.017	0.012	0.04	0.021	0.015	0.026	0.013	0.009

4.7.5. Essential elements in pomace of Citrus germplasm

4.7.5.1. Nitrogen (ppm)

The data regarding pomace nitrogen content as shown in Table No 4.5.10 revealed that the Sikkim mandarin at ripened stage collected from 1400-1600 m altitude had highest value of 4802.22 ppm amongst all the interactions. Whereas the least value was recorded for Sikkim mandarin collected at mature green stage from 1200-1400 m altitude (2870.45 ppm).

On the other hand, pommelo pomace was recorded with 4607.33 ppm nitrogen content at 800-1000 m altitude and 2969 ppm at >1600 m altitude in mature green stage as the highest and lowest. In addition, rough lemon was found with 4466.67 ppm (1200-1400 m altitude) as the maximum nitrogen content and least being occupied by 3033.33 ppm (800-1000 m altitude) in mature green stage.

There was no uniformity of increasing nitrogen content with increased altitude in all the germplasm. Moreover, ripened fruit shows much higher peel ash content as compared to mature green stage irrespective of the different germplasm and stages.

4.7.5.2 Phosphorus (ppm)

Three factor analysis of variance is shown in Table No. 4.5.10. Pommelo was found to be superior having highest pomace phosphorus content up to (280 ppm) at an altitude range of >1600 m altitude at ripened stage followed by 250.33 ppm at altitude >1600 m altitude.

In Sikkim mandarin, pomace phosphorus was recorded up to 245 ppm (800-1000 m altitude) in the ripened stage, whereas in rough lemon it was 238.67 ppm at altitude range (800-1000 m altitude) which was at par with 237 ppm (1200-1400 m) and 236.67 ppm (>1600 m altitude) in the ripened stage.

The lowest pomace phosphorus content during the experiment was observed in rough lemon (126.67 ppm) at 1000-1200 m altitude in mature green stage.

The mature green stage of pommelo showed increasing phosphorus content with increase in altitude. Whereas the rest of germplasms donot have uniform pattern. In all the cases, ripened stage of the fruit was recorded with higher much higher phosphorus content than the mature green stage irrespective of germplasm and altitude.

4.7.5.3 Potassium (ppm)

The pomace moisture as revealed from the three-way interaction of germplasm X altitude X stage of maturity (Table No. 4.5.10). Pomace of pommelo was observed to have 2451.67 ppm at >1600 m altitude at ripened stage whereas the lowest was recorded for mature green rough lemon (1350 ppm) collected from 1200-1400 m altitudes.

Furthermore, 2360 ppm (1000-1200 m altitude) in rough lemon showed the next highest potassium content. However, it was found to be at par with 2252 ppm (1400-1600 m altitude) in the ripened stage.

Moreover, Sikkim mandarin resulted to 2244.33 ppm at 800-1000 m altitude as the maximum potassium content and was at par with 2215.78 ppm (1400-1600 m altitude) in the ripened stage.

Increasing altitude had increased pomace potassium content in pommelo, whereas rough lemon and Sikkim mandarin donot show such trend at mature green as well as ripened stage.

Comparing the two stages showed ripened stage with much higher potassium content than the mature green stage.

Table 4.5.10: Nitrogen, phosphorus and potassium content (ppm) in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Nitrogen		Phosphorus			Potassium		
		Mature green	Ripened	Mature green		Ripened	Mature green		Ripened
Sikkim mandarin	800-1000	3,044.56	3,750.00	201.11		245.00	2050.00		2244.33
	1000-1200	3,603.33	3,633.22	210.00		235.00	1533.33		1844.47
	1200-1400	2,870.45	3,675.67	176.89		235.00	1400.00		1441.11
	1400-1600	3,375.78	4,802.22	191.45		226.33	1607.78		2215.78
	>1600	2,903.44	3,508.89	211.11		244.00	1671.11		1707.78
Pommelo	800-1000	3,420.00	4,607.33	190.33		201.67	1540.00		1966.67
	1000-1200	3,671.67	4,191.67	196.00		206.67	1933.33		2033.33
	1200-1400	3,616.00	3,647.00	196.67		210.00	1976.67		2100.00
	1400-1600	3,436.33	3,618.00	235.00		235.00	2070.00		2300.00
	>1600	2,969.00	3,657.33	250.33		280.00	2400.00		2451.67
Rough lemon	800-1000	3,033.33	3,983.33	155.33		238.67	1486.67		1940.00
	1000-1200	3,566.67	4,300.00	126.67		188.33	2150.00		2360.00
	1200-1400	3,666.67	4,466.67	188.33		237.00	1350.00		2133.33
	1400-1600	4,033.33	4,433.33	190.00		219.67	1850.00		2252.00
	>1600	3,866.67	4,233.33	197.00		236.67	2166.67		2166.70
Factors	**CD (5%)**	**SE(d)**	**SE(m)**	**CD (5%)**	**SE(d)**	**SE(m)**	**CD (5%)**	**SE(d)**	**SE(m)**
Factor(G)	**34.97**	**17.48**	**12.36**	**3.33**	**1.67**	**1.18**	**35.63**	**17.81**	**12.59**
Factor(A)	**45.15**	**22.57**	**15.96**	**4.30**	**2.15**	**1.52**	**46.0**	**22.99**	**16.26**
Factor(S)	**28.55**	**14.27**	**10.09**	**2.72**	**1.36**	**0.96**	**29.09**	**14.54**	**10.28**
Interaction (GXAXS)	**110.59**	**55.27**	**39.09**	**10.54**	**5.27**	**3.72**	**112.68**	**56.32**	**39.83**

4.7.5.4 Calcium (ppm)

The highest calcium content was recorded in pomace of ripened rough lemon followed by Sikkim mandarin and pommelo. Rough lemon grown at 1200-1400 m was recorded with 293.33 (ppm) calcium content and was followed by (273.54 ppm) in the sample collected at ripened stage from 1000-1200 m altitude, (rough lemon) as shown in Table 4.5.11.

Furthermore, Sikkim mandarin was recorded with 192.29 ppm (>1600 m altitude) in ripened stage being next highest after rough lemon, followed by 186 ppm (1000-1200 m altitude) in ripened stage. In pommelo, highest calcium content was 182.49 ppm (>1600 m altitude) in the ripened stage, followed by 171.81 ppm (1400-1600 m altitude) in ripened stage.

Pommelo was found to have followed increasing trend in calcium content with increasing altitude. However, Sikkim mandarin and rough lemon was not in uniformity with increasing altitude. Likewise, pomace calcium content was also found much higher for ripened fruit than the mature green stage irrespective of altitude and germplasm.

4.7.5.5 Magnesium (ppm)

The three way interactions of germplasms, altitude and the stage of maturity (Table No 4.5.11), depicts the presence of highest magnesium (643.18 ppm) in ripened stage of Sikkim mandarin grown at 1000-1200 m altitude as compared to all other interactions, though it was followed by Sikkim mandarin at ripened stage at altitude 1200-1400 m altitude with magnesium content 585.96 ppm.

The next highest was 584.06 ppm (1400-1600 m altitude) in the ripened stage in rough lemon which was followed by 544.33 ppm (1200-1400 m altitude) in the ripened stage. On the other hand, the lowest was 323.67 ppm (1000-1200 m altitude) in the mature green stage.

In addition, pommelo was recorded with 435.74 ppm (1400-1600 m altitude) as the highest which was followed by 407.82 ppm (>1600 m altitude) in the ripened stage.

There was increasing trend for all the germplasm with increasing altitude. In all the cases, ripened fruit peel had higher magnesium than the mature green stage irrespective of germplasms and altitude.

4.7.5.6 Sulphur (ppm)

The three way interactions of germplasms, altitude range and the stage of maturity (Table 4.5.11), depicts the presence of highest Sulphur (23.25 ppm) in ripened stage of pommelo grown at 1200-1400 m as compared to all other interactions, followed up by (Sikkim mandarin) at ripened stage at altitude 1400-1600 m altitude with sulphur content 21.96 (ppm) and was at par with 20.68 ppm (1200-1400 m altitude) in the ripened stage.

In addition, 13.30 ppm (800-1000 m) altitude in the mature green stage was recorded as the lowest sulphur content.

Rough lemon on the other hand showed 21.33 ppm (>1600 m altitude) as the highest sulphur content which was followed by 19.69 ppm (1200-1400 m) altitude in the ripened stage.

There was no increasing trend for all the germplasm with increasing altitude. In all the cases, ripened fruit peel had higher sulphur content than the mature green stage irrespective of germplasms and altitude.

Table No 4.5.11: Calcium, magnesium and sulphur content in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Calcium		Magnesium		Sulphur	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	150.95	168.11	386.67	320.62	14.60	19.35
	1000-1200	151.79	186.00	378.33	307.00	12.44	16.22
	1200-1400	102.26	171.68	390.00	413.33	14.12	20.68
	1400-1600	141.04	183.60	380.67	358.00	16.00	21.96
	>1600	150.04	192.29	405.00	396.67	12.36	21.41
Pommelo	**800-1000**	131.20	154.67	226.57	223.33	13.30	15.15
	1000-1200	155.00	164.19	286.58	243.00	13.70	15.89
	1200-1400	159.67	165.49	353.78	238.51	13.57	23.25
	1400-1600	162.08	171.81	317.33	283.62	13.33	15.95
	>1600	163.00	182.49	390.00	349.61	13.60	16.41
Rough lemon	**800-1000**	159.11	230.32	437.26	360.67	10.71	18.60
	1000-1200	150.90	273.54	419.67	323.67	12.46	18.27
	1200-1400	163.45	293.33	405.00	363.33	13.00	19.69
	1400-1600	154.67	171.02	385.33	341.00	12.80	18.67
	>1600	165.33	203.00	410.42	387.33	16.75	21.33

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	1.62	0.81	0.57	4.72	2.36	1.67	0.55	0.27	0.19
Factor(A)	2.09	1.05	0.74	6.09	3.04	2.15	0.70	0.35	0.25
Factor(S)	1.32	0.66	0.47	3.85	1.93	1.36	0.45	0.22	0.16
Interaction (GXAXS)	5.13	2.56	1.81	14.91	7.45	5.27	1.72	0.86	0.61

4.7.5.7 Iron (ppm)

The data regarding pomace iron as shown in Table No 4.5.12 revealed that the pommelo at ripened stage collected from 1400-1600 m altitude had highest value of 1.93 ppm amongst all the interactions. Whereas the least value was recorded for rough lemon collected at mature green stage from 800-1000 m altitude (0.92 ppm). In addition, rough lemon showed 1.40 ppm (>1600 m altitude) in the ripened stage as the highest iron content. It was in turn at par with 1.13 ppm (1400-1600 m altitude) in the ripened stage and also with 1.22 ppm (1200-1400 m altitude) and 1.21 ppm (>1600 m altitude) in the mature green stage.

On the other hand, Sikkim mandarin pomace was recorded with 1.43 ppm (>1600 m) altitude in the ripened stage as the maximum iron content which was at par with 1.30 ppm in 1200-1400 m altitude in the ripened stage as well as 1.41ppm, 1.37 ppm, 1.27 ppm and 1.23 ppm at 800-1000 m, 1000-1200 m, 1200-1400 m and >1600 m altitude in the mature green stage.

There was increasing trend in pomace iron content with increased altitude in case of rough lemon. However, no such trend was found for Sikkim mandarin and pommelo.

Moreover, ripened fruit shows much higher pomace iron content as compared to mature green stage irrespective of the different germplasm and stages.

4.7.5.8 Manganese (ppm)

The highest manganese content was recorded in pomace of ripened pommelo and Sikkim mandarin at >1600 m altitude with 0.09 ppm followed by rough lemon. Rough lemon grown at >1600 m altitude was recorded with 0.06 ppm manganese content and was at par with 0.05 ppm in the sample collected at mature green stage from > 1600 m altitude as shown in Table 4.5.12.

Furthermore, Sikkim mandarin was recorded with 0.09 ppm (>1600 m altitude) in ripened stage being next higher equivalent to pommelo. In addition, pommelo also showed 0.09 ppm (>1600 m altitude) in ripened stage as the highest manganese content accordingly.

The increase in pomace manganese was in uniformity with increasing altitude for Sikkim mandarin but not for pommelo and rough lemon.

Likewise, pomace manganese content was also found much higher for ripened fruit than the mature green stage irrespective of altitude and germplasm.

4.7.5. 9. Copper (ppm)

The copper content was invariably higher in the samples collected at ripened stage than the mature green one. It was also observed that the copper content was higher at the higher altitude in case of pommelo and rough lemon except for Sikkim mandarin which showed different trend (Table 4.5.12). The copper content was as low as 10.39 ppm in mature green stage Sikkim mandarin collected from 800-1000 m altitude and as high as 24.92 ppm in ripened pommelo collected from > 1600 m altitude. The samples collected at ripened stages in pommelo showed highest copper content which was estimated to the tune of 24.92 ppm at >1600 m altitude. Likewise, in rough lemon it was 21.91 ppm (>1600 m altitude) and in Sikkim mandarin was 19.36 ppm (800-1000 m altitude). There is no uniformity with increasing altitude in all the germplasm. However ripened stage showed much higher copper content than the mature green stage.

Table No 4.5.12: Iron, manganese and copper content (ppm) in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Iron		Manganese		Copper	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	1.10	1.41	0.01	0.02	10.39	19.36
	1000-1200	1.12	1.37	0.01	0.03	13.00	14.76
	1200-1400	1.27	1.30	0.01	0.06	14.05	15.59
	1400-1600	1.02	1.10	0.02	0.08	11.56	13.02
	>1600	1.23	1.43	0.03	0.09	13.08	16.05
Pommelo	800-1000	1.49	1.83	0.01	0.02	15.74	16.33
	1000-1200	1.02	1.73	0.02	0.03	13.85	15.67
	1200-1400	1.18	1.83	0.01	0.02	15.29	19.95
	1400-1600	1.23	1.93	0.01	0.01	14.24	18.00
	>1600	1.19	1.87	0.03	0.09	19.00	24.92
Rough lemon	800-1000	0.92	1.00	0.04	0.04	10.87	17.80
	1000-1200	0.93	1.01	0.02	0.04	14.44	16.21
	1200-1400	1.00	1.02	0.03	0.03	16.00	19.67
	1400-1600	1.13	1.21	0.04	0.04	16.80	17.82
	>1600	1.22	1.40	0.05	0.06	12.45	21.91

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	0.09	0.04	0.03	0.004	0.002	0.002	0.596	0.298	0.211
Factor(A)	0.11	0.05	0.04	0.006	0.003	0.002	0.769	0.384	0.272
Factor(S)	0.08	0.03	0.02	0.004	0.002	0.001	0.486	0.243	0.172
Interaction (GXAXS)	0.29	0.13	0.09	0.014	0.007	0.005	1.884	0.942	0.666

4.7.5.10. Molybdenum (ppm)

The molybdenum in the pomace of pommelo fruit was as high as 0.80 ppm in the ripened samples collected from 800-1000 m and >1600 m altitude which was at par with 0.72 ppm, 0.73 ppm, 0.79 ppm in 1000-1200 m, 1200-1400, 1400-1600 m altitude in the ripened stage as well as 0.70 ppm, 0.67 ppm at 800-1000 m and 1000-1200 m altitude in the mature green stage as shown in three-way combination (Table No 4.5.13).

In regard to Sikkim mandarin, molybdenum content was as high as 0.68 ppm in the ripened samples collected from 1200-1400 m altitude which was at par with 0.59 ppm (1000-1200 m altitude) , 0.63 ppm (>1600 m altitude) in the ripened stage as well as 0.59 ppm (1000-1200 m altitude) and 0.53 ppm (1200-1400 m altitude) altitude in the mature green stage.

Likewise, rough lemon was observed with maximum value of 0.60 ppm at 1000-1200 m altitude at ripened stage. It was in turn at par with 0.56 ppm, 0.50 ppm, 0.57 ppm,0.59 ppm at 800-1000 m, 1200-1400 m, 1400-1600 m ,>1600 m altitude in the ripened stage as well as 0.46 ppm, 0.50 ppm, 0.49 ppm, 0.49 ppm, 0.57 ppm at 800-1000 m, 1000-1200 m, 1200-1400 m, 1400-1600 m and >1600 m altitudes in the mature green stage and the minimum values of 0.46 ppm at 800-1000 m altitude at mature green stage.

There is no uniformity with regards to increase in altitude for all the germplasm. However, ripened stage showed much higher molybdenum content than the mature green stage irrespective of the germplasm and altitude.

4.7.5.11 Boron (ppm)

The boron content of fruit pomace was found to be highest (1.95 ppm) at the ripened stage of Sikkim mandarin grown at an altitude of 1200-1400 m altitude compared to other germplasms grown at different altitudes (Table 4.5.13). On the other hand, rough lemon had the highest pomace boron content to tune of 1.85 ppm at 1200-1400 m altitude followed by pommelo i.e. 1.80 ppm collected from 1400-1600 m altitude at ripened stage. Lowest peel boron content was 1.10 ppm at mature green pommelo collected from 800-1000 m altitude. The pomace boron content was comparatively higher for ripened fruit than that of mature green fruit irrespective of the altitude and stages. However, there is not trend of increase in boron content with increasing altitude in all the germplasm and stages.

4.7.5.12 Zinc (ppm)

Among the three factor interactions, the interaction at altitude >1600 m altitude of pommelo at ripened stage was found to have highest zinc content (1.57 ppm) which was followed by pommelo at ripened stage at 1200-1400 m altitude with 1.39 ppm of zinc content (Table 4.5.13).

The next highest was Sikkim mandarin (0.80 ppm) at >1600 m altitude in the ripened stage which was followed by 0.66 ppm (>1600 m altitude) in the mature green stage.

On the other hand, rough showed 0.61ppm (800-1000 m altitude) as the highest in the ripened stage which was at par with 0.53 ppm (1200-1400 m altitude) in the ripened stage.

Comparison of two stages revealed ripened stage with much higher zinc content than the mature green stage irrespective of the germplasm and stage. However, there is no increasing trend of zinc with increasing altitude in all the germplasm.

Table No 4.5.13: Molybdenum, boron, zinc content (ppm) in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude	Molybdenum		Boron		Zinc	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	0.36	0.52	1.27	1.86	0.35	0.97
	1000-1200	0.59	0.59	1.57	1.93	0.29	0.57
	1200-1400	0.54	0.68	1.77	1.95	0.59	0.75
	1400-1600	0.34	0.48	1.67	1.68	0.35	0.80
	>1600	0.40	0.63	1.41	1.47	0.66	1.00
Pommelo	**800-1000**	0.70	0.80	1.10	1.11	0.30	1.11
	1000-1200	0.67	0.72	1.11	1.57	0.40	1.20
	1200-1400	0.60	0.73	1.13	1.73	1.24	1.39
	1400-1600	0.62	0.79	1.53	1.80	0.26	1.20
	>1600	0.64	0.80	1.71	1.73	0.51	1.57
Rough lemon	**800-1000**	0.46	0.56	1.25	1.33	0.41	0.61
	1000-1200	0.50	0.60	1.37	1.47	0.29	0.42
	1200-1400	0.49	0.50	1.40	1.85	0.53	0.42
	1400-1600	0.49	0.57	1.11	1.21	0.16	0.73
	>1600	0.57	0.59	1.20	1.49	0.37	0.48

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE (m)
Factor(G)	**0.05**	**0.02**	**0.02**	**0.06**	**0.03**	**0.02**	**0.03**	**0.02**	**0.01**
Factor(A)	**0.06**	**0.03**	**0.02**	**0.07**	**0.04**	**0.03**	**0.04**	**0.02**	**0.02**
Factor(S)	**0.04**	**0.02**	**0.01**	**0.05**	**0.02**	**0.02**	**0.03**	**0.01**	**0.01**
Interaction (GXAXS)	**0.15**	**0.08**	**0.05**	**0.18**	**0.09**	**0.06**	**0.11**	**0.05**	**0.04**

4.7.6 Trace element in citrus pomace

4.7.6.1 Aluminium (ppm)

Three factor analysis of variance is shown in Table No. 4.5.14. Pommelo was found to be superior having highest aluminum up to 1.67 ppm at an altitude range of 1400-1600 m, >1600 m altitude at ripened stage and was followed by 1.63 ppm ppm at 1200- 1400 m altitude at ripened stage.

In Sikkim mandarin, aluminum was recorded up to 1.52 ppm (1200-1400 m altitude) in ripened stage. Whereas in rough lemon, 1.57 ppm (800-1000 m altitude) in the ripened stage was found with highest aluminum content.
The lowest aluminum content during the experiment was observed in rough lemon (1.01 ppm) at >1600 m altitude in mature green stage.

There was pattern of increase in pomace aluminum with respect to increasing altitude in pommelo (mature green stage) whereas the rest of the germplasm do not follow any trend. Pomace aluminum was found to be highest for ripened fruit than the mature green stage irrespective of the germplasms and altitude.

However, the highest pomace aluminum in Sikkim mandarin and rough lemon germplasm was observed at 1200-1400 m altitude except for pommelo at 800-1000 m altitude in ripened stage. In all the cases, ripened stage of the fruit was recorded with higher peel aluminum content than the mature green stage.

4.7.6.2 Cobalt (ppm)

The cobalt in the pomace of Sikkim mandarin fruit was as high as 0.47 ppm in the mature green samples collected from 1200-1400 m altitude which was closely followed by 0.35 ppm at 1000-1200 m altitude in mature green stage and that of the lowest value was recorded in Sikkim mandarin (0.09 ppm) at 800-1000 m altitude in mature green stage as shown in three-way combination (Table No 4.5.14).

In regard to pommelo, cobalt content was as high as 0.22 ppm in the ripened and mature green samples collected from 1200-1400 m altitude. The lowest record was 0.10 ppm (800-1000 m, >1600 m) altitude in mature green stage.

Likewise, rough lemon was observed with maximum value of 0.30 ppm at >1600 m altitude at ripened stage and the minimum values of 0.10 ppm at 1000-1200 m, 1400-1600 m, >1600 m altitude at mature green stage. Further there is no uniformity with increasing altitude with respect to all germplasm. Likewise, pomace cobalt content was also found much higher for ripened fruit than the mature green stage irrespective of altitude and germplasm.

4.7.6.3 Sodium (ppm)

The highest pomace sodium content was recorded in Sikkim mandarin followed by pommelo and rough lemon. There was no trend of increase in sodium content with increasing altitude for all the germplasm in both the stages. The pomace sodium content was also found to be much higher for ripened fruit than the mature green stage irrespective of germplasm and altitude.

In Sikkim mandarin pomace sodium content was highest i.e. 19.67 ppm at >1600 m altitude at ripened stage which was found to be at par with 19 ppm (1200-1400 m altitude), 18.33 ppm (1400-1600 m altitude) in ripened stage and 19.33 ppm (>1600 m altitude) in the mature green stage (Table 4.5.14).

Likewise, in pommelo and rough lemon, it was 19.33 ppm (1200-1400 m altitude) and 18.00 ppm (>1600 m altitude) as the highest. However, Sikkim mandarin showed the maximum and rough lemon with the minimum amongst all the germplasm.

Table No 4.5.14: Aluminium, cobalt, sodium content (ppm) content in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Aluminium		Cobalt		Sodium	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	1.26	1.20	0.09	0.13	11.02	12.74
	1000-1200	1.13	1.13	0.10	0.35	14.33	16.33
	1200-1400	1.37	1.52	0.13	0.47	13.88	19.00
	1400-1600	1.37	1.22	0.18	0.27	17.33	18.33
	>1600	1.17	1.27	0.19	0.11	19.33	19.67
Pommelo	800-1000	1.11	1.30	0.10	0.15	11.35	16.67
	1000-1200	1.17	1.17	0.13	0.21	15.33	18.24
	1200-1400	1.17	1.63	0.22	0.22	19.00	19.33
	1400-1600	1.40	1.67	0.13	0.17	12.03	14.33
	>1600	1.43	1.67	0.10	0.16	18.00	18.33
Rough lemon	800-1000	1.47	1.57	0.11	0.25	10.06	12.33
	1000-1200	1.52	1.53	0.10	0.21	13.83	17.69
	1200-1400	1.08	1.20	0.12	0.27	14.33	14.97
	1400-1600	1.12	1.10	0.10	0.16	17.67	18.00
	>1600	1.01	1.04	0.10	0.30	15.09	16.67

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD(5%)	SE(d)	SE(m)
Factor(G)	0.094	0.047	0.033	0.011	0.006	0.004	0.66	0.33	0.24
Factor(A)	0.123	0.061	0.043	0.014	0.007	0.005	0.86	0.43	0.30
Factor(S)	0.078	0.039	0.027	0.009	0.005	0.003	0.54	0.27	0.19
Interaction (GXAXS)	0.302	0.149	0.106	0.035	0.018	0.012	2.11	1.05	0.74

4.7.6.4 Lithium (ppm)

The highest pomace lithium content was recorded in Sikkim mandarin followed by pommelo and rough lemon. There was no pattern of increase in lithium content with regards to altitudinal increase. The pomace lithium content was also found to be much higher for ripened fruit than the mature green stage irrespective of germplasm and altitude (Table 4.5.15).

In Sikkim mandarin pomace lithium content was highest i.e. 0.97 ppm at 1400-1600 m altitude at ripened stage. Likewise, in pommelo and rough lemon, it was 0.91 ppm (1000-1200 m altitude) and 0.59 ppm (>1600 m altitude) as the highest. However, Sikkim mandarin showed the maximum amongst all the germplasm.

4.7.6.5 Barium (ppm)

The pomace barium as revealed from the three-way interaction of germplasm x altitude x stage of maturity (Table No. 4.5.15). Pomace of Sikkim mandarin was observed to have 59.19 ppm at 1000-1200 m altitude at mature green stage as the highest value whereas the lowest was recorded for mature green pommelo (17.33 ppm) collected from 1400-1600 m altitude. Increasing altitude do not follow trend for increasing barium content in all the germplasm.

4.7.6.6 Nickel (ppm)

The result of nickel content clearly illustrates altitude (800-1000 m altitude) of pommelo at ripened stage found to have highest nickel content i.e. 0.68 (ppm) which was found to be at par with 0.60 ppm (1200-1400 m altitude), 0.60 ppm (1400-1600 m altitude) and 0.64 ppm (>1600 m altitude) in the ripened stage (Table 4.5.15).

In addition, Sikkim mandarin (0.63 ppm) showed the next highest at >1600 m altitude in the ripened stage which was in turn at par with 0.56 ppm (>1600 m altitude) in the mature green stage.

Further rough lemon was found with 0.61 ppm (1000-1200 m) altitude in the ripened stage which was followed by 0.52 ppm (1200-1400 m altitude) in the ripened stage.

On the other hand, ripened stage showed much higher nickel content than the mature green stage irrespective of the different germplasm and altitude. Likewise, comparison of altitude with germplasm showed there is no trend of increasing nickel with increase in altitude in all the germplasm.

Table No. 4.5.15. Lithium, barium, nickel content (ppm) in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude	Lithium		Barium		Nickel	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	0.50	0.70	47.67	48.25	0.32	0.42
	1000-1200	0.38	0.53	59.19	55.67	0.31	0.39
	1200-1400	0.46	0.81	46.00	41.67	0.35	0.36
	1400-1600	0.35	0.97	42.00	44.00	0.43	0.54
	>1600	0.44	0.48	42.33	45.00	0.56	0.63
Pommelo	800-1000	0.42	0.48	13.33	10.20	0.57	0.68
	1000-1200	0.76	0.91	14.67	13.56	0.44	0.53
	1200-1400	0.63	0.71	16.00	13.59	0.57	0.60
	1400-1600	0.38	0.50	17.33	15.00	0.54	0.60
	>1600	0.50	0.59	17.33	13.67	0.54	0.64
Rough lemon	800-1000	0.31	0.42	31.76	39.75	0.36	0.46
	1000-1200	0.34	0.39	33.00	40.00	0.46	0.61
	1200-1400	0.35	0.33	31.73	37.52	0.47	0.52
	1400-1600	0.31	0.41	31.33	33.00	0.38	0.38
	>1600	0.44	0.59	33.33	33.92	0.32	0.34

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	0.02	0.01	0.01	1.28	0.64	0.45	0.02	0.01	0.01
Factor(A)	0.03	0.01	0.01	1.65	0.83	0.58	0.03	0.02	0.01
Factor(S)	0.02	0.01	0.01	1.06	0.52	0.37	0.02	0.01	0.01
Interaction (GXAXS)	0.06	0.03	0.02	4.05	2.02	1.43	0.08	0.04	0.03

4.7.6.7 Silicon (ppm)

The silicon content in pomace was highest in Sikkim mandarin collected from 1200-1400 m (0.55 ppm) at ripened stage which was statistically at par with 0.49 ppm, 0.47 ppm at 1000-1200 m altitude, 1400-1600 m altitude in the ripened stage as well as in the mature green stage with 0.43 ppm, 0.53 ppm at 1000-1200 m, 1200-1400 m altitude (Table 4.5.16).

Furthermore, pommelo was found with 0.19 ppm (>1600 m altitude) in the ripened stage as the highest silicon content. On the other hand, rough lemon was found with 0.49 ppm (1200-1400 m altitude) in the ripened stage as the highest silicon content. Whereas the least (0.11 ppm) at 800-1000 m altitude was found in pommelo fruit.

Moreover, mature green stage was estimated with 0.53 ppm in Sikkim mandarin samples collected from (1200-1400 m altitude) and that of the lowest was in the samples of pommelo (0.11 ppm) collected from 800-1000 m.

There was no pattern of increase in pomace silicon content with respect to increasing altitude in all the germplasms. Silicon content in pomace was found to be highest for ripened fruit than the mature green stage irrespective of the germplasms and altitude.

4.7.6.8 Iodine (ppm)

Three factor analysis of variance is shown in Table No. 4.5.16. Rough lemon was found to be superior having highest iodine content up to 1.77 ppm at an altitude range of 1000-1200 m at ripened stage followed by 1.31 ppm at >1600 m altitude in the ripened stage.

In Pommelo, iodine content was recorded up to 1.46 ppm (800-1000 m altitude) in ripened stage which was at par with 1.43 ppm (1200-1400 m altitude), 1.45 ppm (>1600 m altitude) in the ripened stage and also with 1.32 ppm (1200-1400 m altitude) in the mature green stage. Whereas in Sikkim mandarin it was 1.27 ppm at altitude range >1600 m which was at par with 1.17 ppm, 1.13 ppm, 1.26 ppm at 1000-1200 m, 1200-1400 m and 1400-1600 m altitude in the ripened stage.

The lowest iodine content during the experiment was observed in Sikkim mandarin (0.84 ppm) at 800-1000 m altitude in mature green stage.

The mature green as well as ripened stage of Sikkim mandarin showed increasing iodine content with increase in altitude. Whereas the rest of germplasms donot have uniform pattern. However, the highest pomace iodine content in all the germplasm was observed at highest altitude i.e. >1600 m altitude. In all the cases, ripened stage of the fruit was recorded with higher pomace iodine content than the mature green stage.

4.7.6.9 Cerium (ppm)

The maximum pomace cerium (0.26 ppm) was recorded in Sikkim mandarin samples collected at 1400-1600 m altitude in ripened stage. It was also found to be at par with 0.21 ppm (>1600 m) altitude in the ripened stage and 0.21 ppm (1400-1600 m altitude) in the mature green stage (Table 4.5.16).

Likewise, rough lemon was recorded with 0.19 ppm at 800-1000 m, 1200-1400 m altitude altitude as the highest value at ripened stage. Moreover, pommelo was also found with 0.17 ppm (>1600 m altitude) in the ripened stage as the highest which was found to be at par with 0.15 ppm (800-1000 m altitude) in the ripened stage and in mature green stage with 0.14 ppm (1200-1400 m) and 0.11 ppm (1400-1600 m). The cerium content was not in uniformity with increasing altitude in all the germplasm. The pomace cerium content was also found to be higher for ripened fruit than that of mature green stage fruit.

Out of all the 30 combination, 0.26 ppm in Sikkim mandarin at 1400-1600 m altitude in ripened stage was the highest recorded value. Whereas the least was found in rough lemon at 1000-1200 m altitude (0.07 ppm).

Table 4.5.16. Silicon, iodine, cerium content (ppm) in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Silicon		Iodine		Cerium	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	**800-1000**	0.31	0.37	0.84	0.87	0.15	0.19
	1000-1200	0.43	0.49	0.88	1.13	0.12	0.14
	1200-1400	0.53	0.55	0.96	1.17	0.09	0.15
	1400-1600	0.41	0.47	1.01	1.26	0.21	0.26
	>1600	0.38	0.39	1.08	1.27	0.17	0.21
Pommelo	**800-1000**	0.11	0.13	1.13	1.46	0.10	0.15
	1000-1200	0.16	0.18	1.14	1.27	0.09	0.10
	1200-1400	0.13	0.17	1.32	1.43	0.14	0.09
	1400-1600	0.14	0.16	1.02	1.02	0.11	0.06
	>1600	0.14	0.19	1.09	1.45	0.10	0.17
Rough lemon	**800-1000**	0.34	0.39	1.10	1.23	0.12	0.19
	1000-1200	0.41	0.48	1.30	1.77	0.07	0.09
	1200-1400	0.46	0.49	1.23	1.28	0.11	0.19
	1400-1600	0.43	0.46	0.86	1.01	0.13	0.15
	>1600	0.30	0.32	1.10	1.31	0.11	0.16

Factors	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)	CD (5%)	SE(d)	SE(m)
Factor(G)	**0.04**	**0.02**	**0.02**	**0.05**	**0.03**	**0.02**	**0.02**	**0.01**	**0.01**
Factor(A)	**0.06**	**0.03**	**0.02**	**0.06**	**0.03**	**0.02**	**0.02**	**0.01**	**0.01**
Factor(S)	**0.04**	**0.02**	**0.01**	**0.04**	**0.02**	**0.01**	**0.01**	**0.01**	**0.01**
Interaction (GXAXS)	**0.13**	**0.07**	**0.05**	**0.16**	**0.08**	**0.06**	**0.06**	**0.03**	**0.02**

4.7.6.10 Silver (ppm)

The highest silver content in pomace was 0.89 ppm of mature green stage in Sikkim mandarin fruits collected from 1200-1400 m altitude. On the other hand, the lowest content was found in pommelo (0.09 ppm) at mature green stage collected from 1400-1600 m altitude (Table 4.5.17). In addition, rough lemon showed 0.18 ppm (1200-1400 m altitude) in mature green stage as the maxium silver content.

The increase in pomace silver content was irrespective of increase in altitude in all the germplasms.

4.7.6.11 Caesium (ppm)

The highest pomace caesium was recorded in rough lemon followed by Sikkim mandarin and pommelo. There was no trend of increase in caesium content with increasing altitude in all the germplasm and stages (Table 4.5.17).

The pomace cesium content was also found to be much higher for ripened fruit than the mature green stage irrespective of germplasm and altitude.

In rough lemon pomace, cesium content was highest i.e. 0.14 ppm at 1200-1400 m altitude at ripened stage. Likewise, in pommelo and Sikkim mandarin, it was 0.09 ppm (800-1000 m and >1600 m altitude) and 0.10 ppm at 1200-1400 m altitude as the highest. However, rough lemon showed the m aximum amongst all the germplasm.

Table No 4.5.17: Silver and caesium (ppm) content in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Silver		Caesium		
		Mature green	Ripened	Mature green		Ripened
Sikkim mandarin	**800-1000**	0.47	0.58	0.03		0.08
	1000-1200	0.70	0.28	0.05		0.06
	1200-1400	0.89	0.51	0.09		0.10
	1400-1600	0.65	0.79	0.06		0.09
	>1600	0.59	0.83	0.03		0.06
Pommelo	**800-1000**	0.18	0.11	0.02		0.09
	1000-1200	0.19	0.12	0.03		0.06
	1200-1400	0.15	0.15	0.02		0.05
	1400-1600	0.09	0.10	0.01		0.03
	>1600	0.18	0.13	0.05		0.09
Rough lemon	**800-1000**	0.13	0.14	0.02		0.06
	1000-1200	0.11	0.11	0.05		0.12
	1200-1400	0.18	0.13	0.07		0.14
	1400-1600	0.13	0.16	0.08		0.12
	>1600	0.10	0.11	0.04		0.06
Factors	**CD (5%)**	**SE(d)**	**SE(m)**	**CD (5%)**	**SE(d)**	**SE(m)**
Factor(G)	**0.07**	**0.04**	**0.03**	**0.09**	**0.004**	**0.003**
Factor(A)	**0.09**	**0.05**	**0.03**	**0.01**	**0.005**	**0.003**
Factor(S)	**0.06**	**0.03**	**0.02**	**0.01**	**0.003**	**0.002**
Interaction (GXAXS)	**0.22**	**0.11**	**0.08**	**0.02**	**0.012**	**0.008**

4.7.7 Heavy metals

4.7.7.1 Lead (ppm)

Pommelo fruit samples collected from 800-1000 m, 1000-1200 m, 1400-1600 m, >1600 m altitude in both ripened and mature green stage was not detected with lead content. Whereas, 1200-1400 m altitude (0.03 ppm) was found both in mature green and ripened stage.

On the other hand, Sikkim mandarin showed 1000-1200 m altitude without lead content. Whereas the 800-1000 m altitude (0.07 ppm) showed the highest (Table 4.5.18).

Further, in rough lemon 0.08 ppm (800-1000 m altitude) and 1200-1400 m altitude in ripened stage holds the highest lead content. Moreover 0.02 ppm (800-1000 m altitude) was found with the lowest lead content.

Comparison among the stages showed ripened stage with higher lead content than the mature green stage irrespective of germplasm and altitude. In addition to that there was no trend found for increase in lead content with increasing altitude.

4.7.7.2. Cadmium (ppm)

The highest cadmium content in pomace was 0.06 ppm in pomace of ripened stage Sikkim mandarin fruit collected from 1400-1600 m altitude and pommelo fruit collected at >1600 m altitude in ripened stage (Table 4.5.18).

Moreover, rough lemon (0.04 ppm) at 1400-1600 m, 800-1000 m altitude holds the highest cadmium content. However, cadmium was not detected in Sikkim mandarin (>1600 m altitude), pommelo (1400-1600 m altitude) and rough lemon (>1600 m altitude) in ripened and mature green stages.

The increase in pomace cadmium was irrespective of increase in altitude in all the germplasms. Further, pomace boron content was much higher for ripened fruit than mature green stage for all the germplasms.

4.7.7.3 Mercury (ppm)

The maximum pomace mercury (0.17 ppm) was recorded in rough lemon samples collected at 800-1000 m altitude in ripened stage as illustrated in (Table 4.5.18). Similarly, in mature green stage maximum value was 0.14 ppm collected from >1600 m altitude.

Likewise, pommelo was recorded with 0.08 ppm at 1400-1600 m altitude as the highest value at ripened stage. In addition, 0.05 ppm at 1200-1400 m, >1600 m altitude in mature green stage was recorded maximum content in mercury.

Moreover, Sikkim mandarin was also found with 0.06 ppm (800-1000 m, 1000-1200 m altitude) as the highest for ripened stage and that of 0.05 ppm (800-1000 m altitude) as the highest in mature green stage.

There was no uniform pattern of increase in pomace mercury with respect to increase in altitude in all the germplasm. The pomace mercury was also found to be higher for ripened fruit than that of mature green stage fruit.

Out of all the 30 combination, 0.17 ppm in rough lemon at 800-1000 m altitude in ripened stage was the highest recorded value. Whereas the least was found in pommelo at 800-1000 m altitude (0.02 ppm) in mature green stage.

4.7.7.4 Uranium (ppm)

The interaction between altitude, germplasm and fruit stages showed ripened stage (Table 4.5.18) showed that the highest uranium content was recorded in the pomace of pommelo ripened samples collected at 1000-1200 m altitude with an estimate of 0.09 ppm followed by rough lemon (0.08 ppm) at 1200-1400 m and Sikkim mandarin (0.05 ppm) at 1000-1200 m altitude, 1200-1400 m altitude and >1600 m altitude. It is interesting to note that the pomace uranium content donot show any trend with respect to increase in altitude.

The comparison between stages for germplasm revealed ripened stage had higher uranium content in pomace than mature green stage.

Table No 4.5.18: Lead, cadmium, mercury, uranium content (ppm) in pomace of citrus germplasm at different altitude and different maturity stages

Germplasm	Altitude (m)	Lead		Cadmium		Mercury		Uranium	
		Mature green	Ripened	Mature green	Ripened	Mature green	Ripened	Mature green	Ripened
Sikkim mandarin	800-1000	0.03	0.07	0.03	0.02	0.06	0.05	0.02	0.02
	1000-1200	0.00	0.00	0.04	0.05	0.03	0.06	0.03	0.05
	1200-1400	0.03	0.01	0.01	0.03	0.04	0.03	0.02	0.05
	1400-1600	0.03	0.01	0.03	0.06	0.04	0.05	0.02	0.03
	>1600	0.02	0.02	0.00	0.00	0.04	0.03	0.04	0.05
Pommelo	800-1000	0.00	0.00	0.04	0.01	0.02	0.03	0.03	0.04
	1000-1200	0.00	0.00	0.04	0.04	0.04	0.05	0.07	0.09
	1200-1400	0.03	0.03	0.03	0.05	0.05	0.05	0.03	0.05
	1400-1600	0.00	0.00	0.00	0.00	0.05	0.04	0.05	0.03
	>1600	0.00	0.00	0.05	0.06	0.06	0.05	0.04	0.06
Rough lemon	800-1000	0.02	0.08	0.03	0.04	0.01	0.03	0.02	0.03
	1000-1200	0.04	0.06	0.03	0.03	0.04	0.05	0.03	0.04
	1200-1400	0.07	0.08	0.01	0.01	0.02	0.05	0.06	0.08
	1400-1600	0.06	0.06	0.03	0.04	0.04	0.05	0.03	0.04
	>1600	0.04	0.04	0.00	0.00	0.02	0.02	0.04	0.05

Factors	CD (5%)	SE(d)	SE(m)	CD(5%)	SE(d)	SE(m)	CD(5%)	SE(d)	SE(m)	CD(5%)	SE(d)	SE(m)
Factor(G)	0.018	0.009	0.007	0.013	0.006	0.005	0.006	0.003	0.002	0.02	0.01	0.01
Factor(A)	0.023	0.012	0.008	0.017	0.008	0.006	0.008	0.004	0.003	0.02	0.01	0.01
Factor(S)	0.014	0.008	0.005	0.011	0.005	0.004	0.005	0.002	0.002	0.01	0.01	0.004
Interaction (GXAXS)	0.056	0.029	0.021	0.041	0.020	0.014	0.019	0.010	0.007	0.05	0.02	0.02

Note: G= Germplasm; A= Altitude and S= Stage

Chapter 5

Discussion

The fruits and vegetables in our daily life are the major constituents of human nutrition which has been immensely used due to the presence of carbohydrates, protein, fats, vitamins and minerals. The peel and pomace of fruits are usually thrown as the waste, though there has been enough scientific emphasis for value addition and nutritional supplement. The huge potential of these fruit waste has been now identified.

5.1. Waste managment

The peel and pomace waste can be exclusively used in food processing industries as by products. From the 1 tonnes of fruit 0.50- 3.00 kg of oil is liberated from the fruit peel. (Makris *et al*., 2007; Helkar *et al*., 2016; Kodagoda and Marapana, 2017).

The waste from fruit peel and pomace has been a major source of enzymes, reducing sugars, furfural, ethanol, proteins, amino acids, carbohydrates, lipids, organic acids, phenols, activated carbon, degradable plastic composites, cosmetics, bio sorbent, resins, medicines, foods and feeds, methane, biopesticides, bio promoters, secondary metabolites, surfactants, fertilizer and other miscellaneous products (Demirbas,2008). Besides that, total phenolic compounds are also found at higher concentration in peel and pulp of fruit than the edible tissue which can be used for anticancer, anti-microbial (pathogens), anti-oxidative and immune-modulatory effects; reduce incidence of cardiovascular diseases (Makris *et al*., 2007).

The essential oil from the peel can be used in various purposes like natural flavor as well as fragrance. Further it can also be applied in food and also in day to day used chemical products and health care industries (Sheng-min *et al*., 2012). Pathak *et al*., 2017 also explained the use of oil in several aspects like perfumes, medical and in chocolate and sweets and alcoholic beverages for improving the shelf life of food. Likewise, alcoholic beverages, confectioneries, soft drinks, perfumes, soaps, cosmetics and household products due to its aromatic flavour and also mask the bitter taste of drugs in product of pharmaceutical company (Njoroge *et al*., 2005).

Citrus peel and pulp, seed are rich in oil which contain active component D-limonene, a monoterpenoid (Reicks and Crankshaw, 1993) which is worth 20 times than juice in terms of their values. D limonene can be used as hand cleaners and thinner. Citrus peel yield 0.5–3.0 kg essential oil/ tonne of fruit (Sattar and Mahmud, 1986).

D-limonene from lemon can be used for improving immunity, depression, skin health and reduce wrinkles (Sharon, 2008).

Citrus fruit is also found to be a good source of fibre, mineral and vitamins which are needed for human growth and development (Amutha *et al*., 2017). Wadhwa and Bakshi (2013) mentioned pomace being used as an animal feed.They are rich in energy, protein, mineral and vitamins and also being used as livestock feed, poultry and fish (Bakshi *et al*., 2016).

The study has been envisaged on three germplasms of citrus *viz*. Sikkim mandarin (*C. reticulata*), Pommelo (*Citrus maxima* Merr.) and rough lemon (*Citrus* *jambhiri* Lush) to determine its proximate analysis, essential oil and their characteristics, pectin content and characteristics and nutrient content.

5.2. Proximate analysis

Fruit weight, diameter and number of segments recorded for all the crops under studies were in agreement with the studies of earlier workers. Fruit weight of pommelo goes up to 1344 g was in agreement with Mani *et al*., 2017 (1055.33 g) and Hazarika *et al*., 2016 (1624.99 g) as shown in Table 4.2.1, chapter 4.

Likewise, fruit weight of Sikkim mandarin was recorded up to 85.33 g at 1400-1600 m altitude which depicts that this altitude is most suited for cultivation. At low altitude i.e. 800-1000 m and 1000-1200 m, the fruit weight was not satisfactory. At lower elevation, the cultivation of Sikkim mandarin was not usually done probably due to low quality fruits and high incidence of pests and diseases. Fruit weight of rough lemon also depicts that 1000 m to 1600 m altitude from msl is the ideal altitude for its cultivation.

Diameter of fruit and No of segment are correspondingly increase or decrease with increase or decrease in fruit weight of all the fruits under the study. TSS of Sikkim mandarin and pommelo were in the range of 10.17- 13.66 °B which shows the quality of fruits (Table 4.2.2, chapter 4). The TSS of Sikkim mandarin was up to 13.66 °B which was in agreement with the findings of Kishore *et al*., 2010 in Sikkim mandarin; Deshmukh *et al*., 2016 (Khasi mandarin); Boudries *et al*., 2012 (mandarin) Mahajan *et al*., 2018 (Kinnow mandarin); Hazarika *et al*., 2016 (pommelo. Moreover, rough lemon was recorded to 8.33 °B as the maximum TSS which was found higher than Mukhim *et al*., 2015 (Assam lemon).

In all the germplasm, TSS content was found to increase with increase in fruit maturity and was due to accumulation of sugar into the fruit through the hydrolysis of starch and increase level of dehydration (Hossain *et al*., 2016).

There was also an increase in TSS at the initial stage of development (maturity)which may be due to increased accumulation of sugar which later gets converted to organic acid. It may be also due to hydrolysis of acid and deposition of polysaccharides (Ram *et al*., 2003).

Further, the rapid increase in TSS may possibly be due to decrease in acidity with the change in maturity stage of fruit (EI-otmani and Coggins,1991).There was difference in TSS in all the germplasm at different altitude was noticed which may be due to variation in time of flowering and difference in fruit maturity (Deshmukh *et al.,*2016).

Titrable Acidity (TA) was found up to 1.20 in Sikkim mandarin collected at 1200-1400 m altitude. In addition, pommelo hold 1.77 at 800-1000 m altitude and rough lemon goes up to 2.27 at 1200-1400 m altitude in the ripened stage (Table 4.2.2, chapter 4) which was found in corroboration with the findings of Bharali *et al*., 2017 (pommelo); Barros *et al*., 2012 (Pera orange), Sharma *et al*., 2015 (grape fruit). But higher than Hangsing *et al*., 2016 (Khasi mandarin); Riaz *et al*., 2015 (Kinnow mandarin).

The acidity of fruit was higher in immature fruit due to the accumulation of organic acid and also due to higher synthesis of acid in the juice vesicle (Ricevuto, 1933). The decrease in acidity in the ripening stage of fruit might be due to utilization of acids rapidly in the respiration process (Rokaya *et al*.,2016). The acidity of fruit was found to be decreasing with the advancement of maturity in all the altitude as well as maximum decrease was found in higher altitude (Bhusal, 2002 and Bastakoti and Gautam, 2007; Thapa and Gautam, 2002).

There was no trend of increasing acid content with respect to varying altitudes in all the germplasm which may be due to different environmental and growing condition as well as different maturity. As the fruits mature and subsequently ripened, organic acids converts to sugar there by reducing the fruit acidity.

Further, vitamin C content was found higher in immature fruit and got declined gradually with the advancement of growth towards maturity which may be due to utilization in certain metabolic process (Mukhim *et al*., 2015). It was also reported by Lee and Kader, 2000; Gillfillan *et al*., 1972 that change in acid and sugar content of the fruit juice with the changes in maturity may also be the reason for variation of vitamin C with the changes in maturity.

In the present study there was increasing pattern of vitamin C for Sikkim mandarin and pommelo with increasing altitude but rough lemon donot follow any pattern (Table No 4.2.1, Chapter 4). The variation of this pattern may be associated with light, temperature, climatic condition in the growing areas (Correia *et al*., 2016)

Analysis of chemical parameters like titrable acidity, TSS: acid, vitamin C, reducing sugar, total sugar, non-reducing sugar depicts good quality of fruits at different altitudes. TSS: acid ratio in the range of (9.72 -11.88) in Sikkim mandarin; pommelo (6.08-9.16) and rough lemon (3.33-4.99) as illustrated in (Table 4.2.2, chapter 4) was in agreement with the finding of Bharali *et al*., 2017 (pommelo); Kishore *et al*., 2012 in mandarin cultivar.

The TSS: acid ratio was found to decrease with the increase in fruit growth and development which may be attributed to higher acid accumulation and decrease in TSS content in the fruit.

Sinclair and Ramsey, 1944 mentioned that there was increase in TSS, decrease in acidity and finally increase in the ratio of TSS to acid which is in conformity with the present research finding. Decrease in TSS: acid ratio might be attributed to relatively higher rates of accumulation of acids coupled with rapid decrease in TSS and also due to relatively higher rates of accumulation of acids coupled with rapid decrease in TSS.

As such there was no trend of increase/decrease in Titrable acidity, TSS: acid with the change in altitudes within the study site. However, there was increasing trend of reducing sugar, total sugar and non-reducing sugar with increasing altitude in Sikkim mandarin.

It was also found that reducing sugar, total sugar and non-reducing sugar were in agreement with the findings of Yadav *et al*., 2014 (mandarin); Kishore *et al*., 2010 and Kishore *et al*., 2012 (mandarin).

There was increase in sugar content in all the germplasm in the study with the advancement of maturity which might be due to depolymerization of polysaccharides and conversion of fruit starch to sugar. Similar trend was also reported in Khasi Mandarin (Deka *et al.*, 2006) and guava (Patel *et al*., 2013).

The variation of total sugar in different altitude was also attributed in the study which might be due to variation of nutrient status particularly potassium (Pandey *et al*., 2004).

The non-reducing sugar was found to increase from mature green stage to full ripened stage which might be associated with breakdown of starch and there after formation of non-reducing sugar (Moneruzzaman *et al*., 2008).

The moisture content is a parameter related to dry matter content usually used as a measure for stability and aspect of susceptibility from infection. The peel and pomace moisture up to the range of (48.12-60.17 % and 30.67-35.52 % for Sikkim mandarin); (32.67-38.03 % and 36-37.67 % for pommelo) and (37.33-41.67 % and 40.67-47.67 % for rough lemon) respectively was found (Table 4.2.4, chapter 4).

The present finding resulted to lower moisture content than Barros *et al.,* 2012 (lima orange, pera orange, Tahiti lime, sweet lime and ponkan mandarin pomace) but higher than Adewole *et al*., 2014 (orange peel).

There was increasing trend of peel and pomace moisture content with increased altitude in Sikkim mandarin though no trend was observed for pommelo and rough lemon.

Low moisture content is associated with increase in storage life and from the research finding it can be assured that probably the fruits at higher altitude may have less storage life.

The crude fat peel and pomace is one of the aspects determining proximate analysis. The fat content in peel and pomace of Sikkim mandarin were (19.07 %, 15.30 %), pommelo (16.29 %, 15 %) and rough lemon (13.20 %, 19.33 %) in ripened stage (Table 4.2.4, chapter 4) which was found to be higher than Ali *et al.,* 2016 (sour orange, sweet orange, grape fruit, lemon peel); Adewole *et al*., 2014 (orange peel); Egbuonu and Osuji, 2016 (*Citrus sinensis* peel); Peace and Happiness *et al*., 2018

(*Citrus maxima* juice and peel) ; Hiri *et al*., 2015 (Maltease orange peel) ;Sharma *et al*., 2013 (Kinnow pomace).

The crude fat content was found to be much higher than reported for maize, soybean oilcake and sunflower meal and hence can be applied in absorption of fat-soluble vitamin and is thus important in diets (Akinhanmi *et al*., 2008) and also contains highest amount of energy.

There was increase in peel and pomace fat content in mature green stage than ripened stage which might be due to conversion of starch to sugar as the fruit ripened.

The Crude fibre in peel and pomace were (27.44 %, 21.76 %) ; (25.47 %, 27.67 %) ; (22.87 %, 27.67 %) in Sikkim mandarin, pommelo and rough lemon (Table 4.2.5, chapter 4) which was higher than some of the findings like Olabinjo *et al*., 2017(orange); Ahmed Abd Elgh-far *et al*., 2016 (orange , lemon); Singh and Raj, 2018 (lemon) ; Gotmare and Gade, 2018 (orange peel) and Ibrahim and Hamed, 2018 (orange, lemon) . However, it corroborates with the finding of Osarumwense *et al*., 2013 (*Citrus sinensis*).

The high fibre has significance in absorption of trace elements in guts and absorption of cholesterol will also get reduced (Le Veille and Sauberlich, 1966). It also helps in reducing coronary heart diseases, hypertension, diabetes, breast and colon cancer (Jimoh *et al*., 2010).

As per Olabinjo *et al*., 2017 the peel of mandarin can be used in processing of foods and livestock feeds as a low-cost dietary supplement for livestock as well as human beings.

Ash content in peel and pomace is an important factor which determines the composition of minerals in fruit (Leung *et al*., 2009). The ash content in the peel and pomace of Sikkim mandarin (27.44 % , 21.04 %) followed by pommelo (24.33 %, 39.67 %) and rough lemon (13 % , 37.67 %) in ripened stage (Table 4.2.5, chapter 4) was found to be higher than Azad *et al*., 2014 (lemon pomace); Elfghar *et al*., 2016 (orange peel); Egbuonu and Osuji, 2016 (*Citrus sinensis* peel) and Gotmare and Gade, 2018 (orange peel); Ahmad *et al*., 2016 (kinnow, fewtrell's early, malta, mousami, grape fruit, eureka lemon peel) and Ibrahim and Hamed, 2018 (orange and lemon peel).

The higher ash content may be due to high concentrations of mineral elements which speed up the metabolic processes and also improves the growth and development (Barker, 1996). High ash content of peel and pomace signifies the use in animal feed (Pradhan and Sharma,2019).

The ash content during the study was found higher in ripened stage than mature green stage which is attributed to the different absorption capacity of the minerals at different stages of development. High ash content is attributed to the high mineral element composition (Adeyemi and Oladiji, 2009).

In regards to crude protein content in peel and pomace, Sikkim mandarin (3.11%, 3.00 %), pommelo (3.12 %, 3.03 %) and rough lemon (2.75 %, 2.69 %) was recorded (Table 4.2.6, chapter 4). The crude protein was however lower than reported by Janati *et al*., 2012 (*Citrus limon*) ; Egbuonu and Osuji, 2016 (*Citrus sinensis* peel); Ahmad *et al*., 2016 (kinnow, fewtrell's ealy, malta, mousami, grape fruit and eureka lemon peel); Narjis *et al*., 2009 (orange peel) ;Ibrahim and Hamdan ,2018 (orange and lemon peel); Peace and Happiness *et al*., 2018 (*Citrus maxima* juice and peel).

Whereas some researcher was in agreement with the study like Uraku, 2015 (*Citrus sinensis*). There was increase in protein content from mature green to ripened stage in all the germplasm. Protein content was found higher in ripened stage than mature green stage in the present research. Low protein content may not be able to supply adequate amino acid required by the body (Bello *et al*., 2008). If at all peel and pomace are to use as an animal feed, protein from these fruit peel and pomace may be supplemented.

Starch content in peel and pomace in pommelo (6.35 %, 6.22 %), Sikkim mandarin (3.40 %, 1.95 %) and rough lemon (2.32 %, 2.75 %) as illustrated in Table 4.2.6, chapter 4 was higher than Wanlapa *et al*., 2015 (Durian, mango) peel.

Peel and pomace phenol up to (244.90 mg/GAE/g, 121.29 mg/GAE/g) in Sikkim mandarin; (124.02 mg/GAE/g, 122.86 mg/GAE/g) in pommelo and (121.01 mg/GAE/g, 134.86 mg/GAE/g) in rough lemon respectively was found during the study (Table No 4.2.7, chapter 4). The content was higher than Elfghar *et al*., 2016 (orange and lemon peel); Shie and Lay, 2013 (*Citrus limon*); Adeline *et al*., 2016 (*Citrus aurantium*); Chanalia *et al*., 2018 (*Citrus limetta*); Boudries *et al*., 2012 (clementine cultivars like St Martin, Cadoux cv, Cheryland cv, Monreal cv, Rocamora, Merme cv and Mandarin).

However, Adewole *et al*., 2014 showed higher phenol content in orange peel than present study.While Ghasemi *et al*., 2009 (*Citrus sinensis* var Washington, *Citrus reticulata* var Ponkan, *Citrus unshiu* var. Mahalli, *Citrus unshiu* var. Sugiyama, *Citrus sinensis* var. Sungin, *Citrus unshiu* var. Ishikawa, *Citrus limon*, *Citrus reticulata* var. Clementine, *Citrus sinensis* var. Valencia, *Citrus aurantium* var. Khosheii, *Citrus reticulata* var. Page, *Citrus paradisi* and *Citrus aurantium)* was in agreement with phenol content . It was interesting to note that the phenol content in peel was higher than that of pomace. Higher phenol in peel is due to higher concentrations of compounds in the outer layer which are more predisposed in the process of forming phenolic compounds (Barros *et al.,* 2012).

The high phenolic content of the fruit is attributed to its strong antioxidant activity. Further the fruit has prospects for *in vivo* studies of medicinal active components and can be used for preparing natural pharmaceutical products owing to high value (Stankovic, 2011).

The phenolic content was found in peel than pomace which may be due to accumulation of phenolic compounds in the dermal tissues of the plant body. Further it helps in protection against UV radiation, defence against pathogen and predators (D'Abrosca *et al.* 2007). The variation in phenol is also due to several factors like genotypic differences, geographic and climatic conditions, harvesting time and implementation of cultural practices (Bayili *et al*., 2011).

High antioxidant activity was found in peel and pomace of all the studied citrus species. The free radical scavenging activity in peel and pomace was in the range of 82.68 % (rough lemon) to 93.33 % (Sikkim mandarin). The pomace registered less free radical scavenging activity in the range of 72.67 % (pommelo) to 95.47 % (Sikkim mandarin) mentioned in Table 4.2.7, chapter 4. As such there is no trend of increasing/ decreasing of antioxidant properties with change in altitude but the present value is much higher than reported by Prakash *et al*., 2016 in *Citrus aurantium*. But was in agreement with Javed *et al*., 2014 (*Citrus reticulata*).

The peel and pomace are rich sources of antioxidant which help in scavenging free radicals and thus helps in unwanted side reaction which can causes cell damage (Balasundram *et al*., 2006). There was increase in antioxidant properties by free radical scavenging activity by DPPH method both in peel and pomace of the germplasm. The increase in antioxidant activity is due to reduction in temperature and exposure of more UV radiation for synthesis of antioxidant compounds (Zargoosh *et al*., 2019).

Further in the present study mature green peel showed higher antioxidant activity than ripened one which is due to the change that occur during the ripening process. During ripening phytohormone ethylene is released and it activate transcription genes which helps in ripening process by synthesis of enzyme and thus no of free radical are formed which detoxify free radical by converting it to harmless substance and hence there is reduction in antioxidant content in the ripening process (Jacob *et al*., 1999).

IC_{50} in peel and pomace was (1.97 mg/ml, 3.83 mg/ml) in Sikkim mandarin; (1.83 mg/ml, 2.14 mg/ml) in pommelo and (2.78 mg/ml, 1.70 mg/ml) in rough lemon respectively (Table 4.2.8, chapter 4). In the present study, IC_{50} value tend to reduce with the maturity of fruit which may be due to metabolism into inactive compounds or increase amount of water, reduction of vitamin C in mature fruit.

Sikkim mandarin showed the clear trend of increasing flavonoid content with increased altitude, though no such trend was found in pommelo and rough lemon. The variation in flavonoid content was due to difference in soil nutrient content, rainfall, photosynthesis rate and light intensity which had a stronger effect on its content (Majuakim *et al*., 2014). The altitude has direct bearing on above parameters.

Further ripened stage tends to decrease in flavonoid content in all the germplasm which is associated with reduction in vitamin C levels (Bocco *et al*., 1998).

5.3. Essential oil and its characterization

The oil yield was up to 2.40 % (Sikkim mandarin), 2.61 % (rough lemon) and 1.79 % (pommelo %) (Table 4.2.9, Chapter 4). The oil yield was higher than reported by Kamal *et al*., 2011 (*Citrus reticulata);* Ahmad *et al*., 2016 (Malta, eureka lemon , mousami , grapefruit , kinnow and fewtrell's early) ; Esfahani and Moradi, 2017 (lime fruit) ; Ghulam *et al*., 2013 (kinnow, musammi and grape fruit) ; Javed *et al*., 2014 (mandarin, mousami grapefruit, malta, tangerine).

Higher oil content of these citrus species points towards the possibility of commercial essential oil extraction. The variation in oil yield may be attributed to presence of gums in peels, difference in albedo and flavedo layers, extraction methods, maturity and stages of harvesting. However lower oil yield may be attributed to some gums being present in the peel. Further water supply in ripening stage also had major influence in oil yield content (Rebey *et al*., 2009).

Oil content was higher at higher elevation for all the studied germplasms. At different altitudes, sunshine exposure, climatic condition is different there by. In addition, higher altitude had shown effects on essential oil production which may be due to different sunshine exposure or may be due to difference in climate which leads to decrease in nutrient absorption (Khorshidi *et al*., 2009).

Comparing the various characterization of oil with the literature resulted to several findings like Evbuomwan *et al*., 2016 (Grapefruit) showed agreement for specific gravity, RI, saponification value but lower with acid value.

Likewise, Bhuyan *et al*., 2015 in khasi mandarin peel, Kashaf *et al*., 2013 in grape fruit, Javed *et al*., 2014 (Mandarin Mousami, Grapefruit, Malta, Tangerine) , Olabanji *et al*., 2016 (orange*)* and Njoku and Evuomwan, 2014 (orange) oil resulted in agreement with the study for specific gravity and RI which showed that the extracted oil is pure. However, Zaker, 2017 in orange was found with higher iodine value and lower acid value.

Ramadan *et al.,*2018 (Valencia) coincides with the RI value but resulted into higher FFA, acid value and lower saponification value, ester value and iodine value. Further Demery *et al*., 2016 (orange oil) mentioned lower acid value, colour value, FFA, saponification value. But higher iodine value, and was in agreement for RI.

Specific gravity was up to 0.87 (pommelo), 0.86 (Sikkim mandarin) and 0.86 (rough lemon) as mentioned in Table 4.2.9, chapter 4).

The result was found in agreement with the finding of Ezejiofor *et al.,* 2011 (sweet orange peel) and Okunowo et *al*., 2013 (grape fruit peel) oil. The present study also reveals the presence of specific gravity withing the range of 0.85 and 0.86 (Codex Alimentarium Commission, 1993). There was no trend of increase/ decrease in this parameter with the change in altitudes within the study site for all the germplasm.

The specific gravity values of all the germplasm were < 1 which indicates that the oil is lighter than water and also being insoluble in water (Fakayode and Abodi, 2018).The refractive index (RI) reported in the present study were similar in both the stages and was within the range of 1.46-1.49 in all the germplasm (Table 4.2.9, chapter 4). RI helps in determining response of oil and bends light. RI value of 1.47 was marked highly pure (Kumar, 2014). Hence the RI from the study can be identified as highly pure. It was in agreement with Ali, 2015 (orange, grape fruit and lemon peel oil); Ghulam *et al*., 2013 (*Citrus reticulata*, *Citrus sinensis* and *Citrus paradisi*); Colecio-Juárez *et al*.,2012 (sweet lime peel oil).

Oil colour is an indicator of heat treatment and can be used to predict the deterioration of quality resulting from heat exposure (Lozano and Ibarz 1997, Shin and Bhowmik 1995). Colour value was up to 1.40 (rough lemon), 1.06 (Sikkim mandarin) and 1.06 (pommelo) as the maximum values. Differences in colour values is due to the difference in pigment type and their concentrations (Awaad *et al*., 1980).

Free fatty acid (FFA) content in Sikkim mandarin (7.83 %), pommelo (7.38 %) and rough lemon (8.27 %) as in Table 4.2.10, chapter 4) was found higher than reported by Khan *et al*., 2013 (sour orange, sweet orange).

It was also interesting to note that FFA was higher in mature green than ripened stage which may be due to higher chlorophyll and water content of the fruit, which ultimately facilitates lipolysis, dilute the antioxidant enzymes and stimulation of growth of the microorganism (Kiritsakis *et al*., 1998).

Essential oil should contain minimum of 6 % FFA for soap making (FAO)/WHO, 2008). The present finding supports the use of essential oil for soap making. However, the value was > 6 %.

The variation in FFA content was due to altitude which was also reported by Freihat *et al*., 2008 .The acid value was up to 12.74 % (pommelo), 8.37% (Sikkim mandarin) and 8.97 % (rough lemon) as in (Table 4.2.11, chapter 4). The oil with high acid value >10 mg/KOH/g of oil is in edible (Barkatullah *et al*., 2012) but they can be used in paint industry owing to outstanding storage life (Zaker, 2017). The effect of altitude on acid value of oil was reported by Ramadan *et al*., 2018.

Oil phenol was up to 8.27 mg/g (pommelo), 8.96 mg/g (rough lemon) Sikkim mandarin (8.19 mg/g) as the highest (Table 4.2.10, chapter 4) in the study which was found in corroboration with the finding of Khan *et al*., 2012 (grape fruit). Pommelo showed the clear trend of increasing phenol with increased altitude, though no such trend was observed in Sikkim mandarin and rough lemon.

Saponification value was highest in rough lemon oil (165.35 mg KOH/g oil) and there was trend of increase or decrease of saponification value with increasing altitude in all the germplasm (Table 4.2.11, chapter 4). The present data shows the high saponification value from oil of these three species than reported so far by Ali, 2015 (orange, grape fruit and lemon peel oil).

The present study revealed presence of higher saponification value which leads to formation of lower molecular weight oxidation products which has higher unsaturated fatty acid and can be used in making soap and shampoos (Audu *et al*., 2013). The high saponification value indicates high fatty acid percentage (Omolara and Dosummu, 2009) and can be used in the industry (Akubugwo and Ugbogu, 2007).

The ester value of Sikkim mandarin, pommelo and rough lemon were as high as 157.54 mg KOH/g oil, 153.86 mg KOH/g oil and 170. 10 mg KOH/ g of oil respectively (Table 4.2.11, chapter 4). The values are higher than reported by Ali, 2015 (orange, grape fruit and lemon peel oil). There was also no trend of increase/ decrease in ester value with increasing altitude in all the germplasm. It was also found that ripened fruit occupies higher ester value than mature green stage.

Peroxide value can be used as a means of lipid oxidation. Peroxide value was up to 8.84 m e q/kg, 31.33 m eq./kg and 17.33 mg eq./kg in pommelo, Sikkim mandarin and rough lemon (Table 4.2.10, chapter 4).

The highest peroxide value in Sikkim mandarin showed that it had undergone primary oxidation compared to pommelo and rough lemon as peroxide value measure the extent to which an oil sample has undergone primary oxidation.

The study also showed that peroxide value exceeds the permitted maximum peroxide value for edible oil i.e. 10 me O_2/kg of oil (FAO/WHO, 1993) in Sikkim mandarin, rough lemon and thus was not found suitable for consumption but can be utilized in industrial application like cosmetics industry i.e. soap making, perfumes and unguents.

The present study found pommelo without any trend of increase/decrease in peroxide value with increasing altitude. However, Sikkim mandarin and rough lemon were following the trend which coincides with the finding of Mousa *et al*., 1996 that may be due to decrease in lipoxygenase enzyme activity as mentioned by Gutierrez *et al.,*1999; Uceda *et al.,*1985.

Iodine value was within the range of 74 g I_2/100g) in rough lemon to 116 g I_2/100g in Sikkim mandarin (Table 4.2.11, chapter 4). This result was supported by Khan, 2013 (sour orange and sweet orange peel oil).

The iodine value from the present study was also within the range of 90 and 130 which means it is semi drying oil which showed that oil of all the germplasm are rich in unsaturated fatty acid and thus had higher amount of double bond (Guner *et al*., 2006) which has application in paint industry as well as varnishes (Nwobi *et al*., 2006). It was also found that higher iodine value showed greater the number of C=C double bonds and thus the oil can be used in making soap (Akinhanmi *et al*., 2009). The iodine value was also found higher in mature green fruit than ripened stage which may be due to the reduction in antioxidant content in the ripening process (Jacob *et al*., 1999).

The thiocyanogen value (T.V) in pommelo (77.87 %), Sikkim mandarin (77.30 %) and rough lemon (75.78 %) as in Table 4.2.11, chapter 4. The result of the study was higher than Ali, 2015 (orange, grape fruit and lemon); Mohammed *et al*., 2014 (melon seed oil). There was increasing trend of thiocyanogen value with increasing altitude for pommelo. However, Sikkim mandarin and rough lemon did not follow any trend. Mature green stage was also found higher compared to ripened stage in all the germplasm. TV is useful in estimating the composition of mixture containing two unsaturated acid which is useful in grading of food oil as the composition helps in stability of food during storage (Knight, 1939).

The study on antioxidant activity by DPPH method revealed up to 82 % (pommelo), 81.71 % (rough lemon) and 80.53 % (Sikkim mandarin) (Table 4.2.10, chapter 4) was found higher than the study conducted by Khan *et al*., 2012 (Jaffa, blood red, grape fruit), Yadav *et al*., 2017 (*Citrus paradisi*).

5.4. GCMS analysis

GCMS analysis of essential oil revealed the presence of (42, 68) compounds in Sikkim mandarin; (41,49) compounds in pommelo and (50, 52) compounds in rough lemon at mature and ripened stage. Dominant component in all the germplasm were α-Thujene ,α -Pinene ,Camphene, Sabinene ,β-Pinene, Myrcene, octanal, Limonene, β-Ocimene, γ-Terpinene, α-Terpinolene, Linalool, Nonanal, Citronellal, Terpinen-4-ol, α-Terpineol, Decanal, Nerol, Neral , Geraniol, Geranial , Germacrene B , Germacrene D, α-Farnesene, β-Farnesene ,γ-Elemene, δ-Elemene , β-Elemene, Cis-Linalool oxide, Trans-Linalool oxide , α-Sinensal and β-Sinensal compounds .

Acetophenone, α-p dimethylsyrene, cis-citral, 2-decyn-ol, transvalenrenyl acetate, ascaridole, ethanone, hordenine, cis-thijopsenic acid, torulosol, ethanone, ascaridole, α-fenchene ,m-cymen-8-ol, α-Murolol, cyclopentane-1,2-dione, β-eudesmol, α-bergamotol compounds were absent in pommelo and rough lemon as compared to Sikkim mandarin

The major compound limonene was as high as 89.46 % and 88.46 % (Sikkim mandarin) at Table 4.3.1, chapter 4; 95.27 % and 94.81 % (pommelo) at Table 4.3.2, chapter 4; 91.62 % and 92.56 % (rough lemon) at Table 4.3.3, chapter 4 in mature green and ripened stages (Table. The present data shows that the high extraction of limonene from the peel of these three species than reported so far (Ascrizzi *et al.*, 2018 in lemon peel oil, Owolabi *et al.*,2018 in lemon peel and leaf, Ayala *et al.*, 2017 and Sikdar *et al.*, 201F6 in orange peel oil

However, higher limonene content was shown by Dharmawan and Kasapis, 2008 (Pontianak oranges, Mosambi and Dalandan peel oil), Jalgaonkar *et al*.,2013 (Soh Sarkar, Pommelo peel oil).

It is noticeable from the research finding that compounds like α-thujene, α-pinene, myrcene, sabinene, octanal, β-ocimene, γ-terpinene, terpinen-4-ol, α-terpineol, linalool decreased in quantity with increasing altitude whereas constituents like camphene, sabinene, limonene, β-pinene increase in quantity with decreasing altitude in ripened and mature green stage of Sikkim mandarin. Moreover in pommelo, α-thujene, myrcene, limonene, β-ocimene, γ-terpinene compounds tend to decrease and sabinene, α-Pinene, linalool, terpinen-4-ol, α-terpineol were found with increase at higher altitude (>1600 m altitude). On the other side, rough lemon showed α-pinene, α-thujene, sabinene, myrcene, γ -terpinene, linalool, nonanal tend to decrease and that of compounds like β-pinene, octanal, limonene increases with increase in altitude.

The variation in the presence of compounds may be due to genotype, plant ecotype, geographic origin, adaptive process and ecological condition (Chalchat *et al*., 1995; Djarri *et al*., 2008; Faidi *et al*., 2014). Further changes in soil and solar radiation may be the other factor in varying altitude which leads to change in the biosynthetic pathway. Hence the change of ecological niches will ultimately change the essential oil compositions as well as components. (Arruda *et al*., 2012).

In addition, nerolidol, cis-limonene oxide, trans-limonene oxide, ascaridole, α-fenchene, 1-Nonanol, m-cymen-8-ol, β-sinensal and trans-α-bergamotene which were found at >1600 m altitude was not observed in low altitude (800-1000 m). In contrast to that some compounds like undecanal, δ-cadinene, 1-nonanal, γ-terpineol acetate, trans-carveol, 2,6-octadien-1-ol were only detected at 800-1000 m altitude in Sikkim mandarin.

But in pommelo o-cymen-5-ol, β-bisabolene, isothujol, β-caryophyllene oxide, (Z) asarone, trans-α-bergamotene, limonen-10-yl-acetate, cis-p-mentha-2,8-dien-1-ol, trans-p-mentha-2,8-dien-1-ol, nerol and nerolidol were detected.

During the study, major effect of altitude was seen on synthesis of compounds in citrus peel. Limonene content was higher at lower elevation and descended as the elevation was increased. camphene was detected in the samples only in the altitude range of 800-1000 m.

Several researchers showed no of compounds being lower than the findings like: Prasad *et al.,* 2016 (grape fruit leaf and rind) showed 42 and 34 compounds; Chun Yan *et al*., 2010 in lemon peel oil showed 55 compounds. But there is researcher like Goyal and Kaushal, 2018 in mandarin oil who mentioned 80 compounds in their research findings.

Taking into account of the research done by others and comparing with the present study in regards to GCMS analysis of oil. We found that limonene is the major constituents derived from the peel of fruits. Whereas, α-Pinene, β-Pinene, sabinene, myrcene, octanal, linalool, were also being considered as major chemical constituents in our study.

Limonene has been considered as a flavouring agent (Sun, 2007) in the code of Fedral Regulation and is considered as safe flavouring agent. It has several applications like curing gastric disorder, antiviral, antimicrobial, sedative, antilithic (Duke, 2001).

Besides that, other compounds had been useful for several properties like anti-inflammatory, anti-bacterial, antifungal, perfumery, flavouring, analgesic, antidepressant, antipsychotic and anxiolytic.

5.5. Analysis of pectin

Pectin extraction from the fruit which are naturally rich in pectin has been a major substitute for commercial pectin which may not be available in all the residing areas and also to get rid of buying for poor farmers. The extracted pectin was subject to characterization of physical (moisture, colour, solubility in cold and hot water, solubility in cold and hot alkali, % yield) and chemical (sugar, organic acid, equivalent weight, methoxyl content, anhydrounic acid, degree of esterification, carbonate free ash, alkalinity as carbonate, ash content). Physical parameters obtained during the present studies were at par with the finding of Kanmani *et al*., 2014; Aina *et al*., 2012.

Sugar and organic acid tend to decrease with the change in maturity. There was also trend of increasing this parameter with increasing altitude for Sikkim mandarin and pommelo. However rough lemon didnot follow the trend.

Pectin yield was highest in Sikkim mandarin peel there was increasing trend with increase in altitude for all the germplasm under study. It was also noticed that the pectin was higher in mature green stage than the ripened stage. It may be due to conversation of pectin into protopectin, sugar and other sorts of constituents which got increased during the ripening process (Rha *et. al*., 2011).

The pectin with high equivalent weight would have high gel forming ability and vice versa. The free acid may also cause the increase or decrease in equivalent weight (Yadav *et al*., 2017).

Equivalent weight values are being applied for calculating anhydrounic acid (AUA) content and degree of esterification (DE). Methoxyl content is one of the important factors which help in controlling the setting time of pectin and gel forming ability of the pectin (Constenla and Lozano, 2003). The spreading and sugar binding capacity of pectin is dependent on pectin methoxyl content (Yadav *et al.,* 2017).

From this study, the extracted pectin samples can be divided into high and low methoxyl pectin (LMP) as the values were > 7% and < 7%. Hence it can be mentioned that the pectin of the study is desirable in terms of quality (Joslyn, 1980).

The Anhydrouronic Acid (AUA) is associated with the purity of the extracted pectin and as per Food Chemical Codex (1996) the AUA determines the purity of oil and it should not be less than 65 % which means that the pectin may have sugar and starch in considerable amount (Ismail *et al*., 2012).

DE in the present study was higher than 50 %, which can be categorized as high methoxyl pectin (HMP). It requires pH of 3 to form gel and are soluble in hot water and contain dextrose which prevents from lumping. It is also seen that DE decreased with increase in maturity although it is also dependent on species, tissue and stages of maturity (Sundar *et al*., 2012). The lower DE during ripening may be due to pectin conversion to protopectin which ultimately increases the sugar and make fruit softer (Bartley and Knee, 1981; Redgwell *et al*., 1997).

The ash content during the present study was in the range of 4.17 % to 8.83 % irrespective of altitude, stages of fruit maturity and species (Table 4.4.3, chapter 4). Ash content should be < 10 % for best gel formation (Ismail, 2012). Hence, the pectin extracted from the fruit peel under study is up to mark.

It also showed that pectin is pure and might having good gelling ability and also indicates mineral present in food (Ahmmed *et al*., 2017).

The variation in these parameters may be due to increase in ash content which is associated with decrease in yield and also indicates that sugar and other constituents increase during the ripening of fruits (Azad *et al*.,2014).

During the present study there was increase in equivalent weight with increase in altitudes in all the fruit crops under study. Likewise, methoxyl content, anyhdrounic acid also follows same trend. It implies that the peel from the higher altitude would be more productive for pectin extraction. The variation in pectin is attributed to the difference in soil characteristics as well as the climatic condition in the different altitudes. pectin is heat sensitive. There is no reason ascribed so far for high altitude for increased pectin.

% yield of pectin during the present study was higher than reported by Aina *et al.,* 2012 in lemon (*C. limon*), Grapefruit, (*C. paradisi*) and Sweet Orange (*C. sinensis)* ;Ahmed and Sikder, 2019 (*C. limon var.* Ginger lemon, Cardamom lemon and China lemon) and Yadav *et al*., 2017 (sweet lime peel) .

The FTIR analysis inferred functional group which helps in determining the bond of pectin. Absorption band was observed to stretching vibration of hydroxyl group (O-H) functional group, C-H stretch as carbohydrate ring and strong C=O bond was found. The strong peak in the range of 1219 cm^{-1} suggest the presence of alcohols, carboxylic acid and esters (Thetsrimuang *et al*., 2011). It is used for the structural characterization of pectin and as such no significant difference was found in the pectin structure amongst all the samples under study.

5.6. Ionome profiling of peel and pomace of citrus germplasm

Plant possesses several minerals which is required for nutritional, medicinal and therapeutic purposes (Rajurkar and Damame, 1998; Chowdhury and Rehman, 2002 and Al- Kharusi *et al*., 2009; Higdon, 2003; Lieberman and Brunning, 2003). About 14 elements are required for human health like N, P, K, Ca, Mg, Na, Cu, Fe, Zn, Mn, Co, Si, Br and Cr. Excess of these elements result to toxicity but deficiency may lead to health problems (Rajangam *et al*., 2001; Aslam *et al*., 2005).

As shown in the results (Table 4.5.1-4.5.18, Chapter 4), the peel and pomace had the abundance with the trend of N > K > Mg > P > Ca > Na > Cu > S > Fe > Bo > Mo > Zn > Li > Co > Mn below the RDA. Elements like P, K Ca, Mg, Cu, Zn,Al, Co, Ba,Ag,Cd, were more in pomace and in peel S, Fe, Mn, Mo, Bo, Zn,Li,Ni.Si,I,Cs, Hg and U were found higher.

Hence, the result showed that the most of the major and minor nutrient are found in pulp and can be a potential source in pharmaceutical industry or in the functional food (designer foods). On the other hand, peel of all the germplasm also showed presence of N K S, Fe, Mn, Mo, Bo, Li, Na, Hg, Ni, U, Si, I in much higher quantity which can be utilized in the preparation of mineral of varying composition.

Nutrient content in citrus peel so far reported higher than the present study was Na, Fe, Ca (Ani *et al*., 2018 in pommelo juice and peel); K, Na, Ca, Fe, Mn, Zn, Pb, Al (Ali *et al*., 2016); Ca, Mg, Mn, Cu, Bo, Zn, Fe (Ozcan *et al*., 2016 in Lemon, Lime, Orange, Grape fruit, orange , mandarin peel).

Czech, 2019 in orange, pommelo, mandarin, lemon, key lime, red grape fruit, green grape fruit, white grape fruit when compared between peel and pomace of citrus germplasm showed that phosphorus, sodium, calcium and iron content was significantly in agreement.

Likewise, K, Ca was in close proximity (Barros *et al*., 2012 in Lima orange, Pera orange Tahiti lime, Sweet lime, Ponkan mandarin). Whereas lower result was shown as P, K, Ca, Mg, Cu, Cd, Na, Ba, Ni (Hong *et al*., 2018 in Cheonhyehyang, Hallabong, Jinjihyang, Hwanggeumhyang, Redhyang, kumquat, Lime, Lemon, Yuzu, orange and Grapefruit).

Comparison among the germplasm with regards to elemental composition showed N, P, Ca, Fe, Ni Mb, Li, Na, Co (Sikkim mandarin); Zn, Mn, Mb, Cu, Ni (pommelo) and Na, Mg, B, Ba, Ca, I (rough lemon) as the maximum content. Hence, higher magnesium in pommelo can be presumed as a means of treating people suffering from hypertension, high blood pressure and asthma.

Further higher zinc showed advantage of people suffering from osteoporosis, arthritis, night blindness. In addition, Sikkim mandarin peel and pomace can be used for anaemia, oxidative stress, bacteria and viruses and rough lemon can be applied as a source of calcium for adults.

Chapter 6

Summary and Conclusion

The present study entitled "Essential oil, pectin and ionome profiling of citrus fruit waste was carried out at Department of Horticulture, Sikkim University, Gangtok to estimate calculate the nutrient content of three citrus germplasms.

Broadly, present work comprises proximate analysis of peel and pomace, essential oil extraction from rind and its characterization, extraction of pectin and its characterization and ionome profiling of peel and pomace of citrus germplasm.

The study was carried out in three germplasm *viz:* Sikkim mandarin (*Citrus reticulata* Blanco), Pommelo (*Citrus maxima* Merr.) and rough lemon (*Citrus jambhiri* Lush).

The fruit samples of all germplasm were collected from five different altitudes in two stages of maturity (mature green and ripened). Standard methods were employed for analysis and estimate of different parameters. The results and major outcomes are summarized as follows:

- Sikkim mandarin was selected for the study for being the most commercial fruit of Sikkim hills. Pommelo and rough lemon were selected being abundantly found, though not commercial.
- The pommelo fruits grown in the hills are grown at different altitudinal gradient showed highest in edaphic characters like non-reducing sugar, peel and pomace crude protein, peel and pomace starch, phenol pomace and

pomace flavonoid as compared to Sikkim mandarin and rough lemon irrespective of altitude and maturity stages.

- Rough lemon was recorded with highest titrable acidity, vitamin C, pomace moisture, peel flavonoid, peel moisture, crude fat, crude fibre, peel ash, pomace ash, peel phenol antioxidant activities in peel and pomace, IC_{50} of peel and pomace.
- Sikkim mandarin showed highest TSS, reducing sugar, total sugar, TSS: acid, peel moisture, crude peel fat, peel ash, peel phenol and pomace IC_{50} in which reducing sugar, peel moisture and peel ash showed trend of increase with increasing altitude. Whereas, rest of the parameter donot follow any trend.
- The physico chemical characterization of oil depicted parameters like specific gravity, acid value, phenol and antioxidant activity (DPPH method) being the maximum in pommelo. Whereas, oil yield, RI, ester value, peroxide value, Iodine value were found to be much higher in Sikkim mandarin. Lastly rough lemon holds color, FFA and saponification value with the maximum value.
- Biochemical parameters that determines the quality of fruit *viz:* TSS, titrable acidity, Vitamin C, reducing sugar, TSS: acid, total sugar, crude peel fat, crude fibre peel, peel phenol, peel flavonoid in Sikkim mandarin was at par with other commercial *Citrus reticulata* fruit of India.
- The peel oil characteristics results for refractive index, highly pure, saponification value, iodine value, FFA, iodine value, acid value showed that though it is highly pure it is not suitable for consumption. However, it has industrial application i.e. soap making, shampoo and paint industry.
- On GCMS analysis of essential oil extracted from peel, 68, 49 and 52 components were detected in Sikkim mandarin, pommelo and rough lemon at

varying altitudes and maturity stage. Limonene content in oil was as high as 95.27 % in pommelo, though its content was not less than other citrus germplasm in Pontianak oranges, Mosambi, Dalandan, Soh Sarkar, Pommelo peel oil in various research findings.

- Other research components were β-Pinene (2.99 %) in pommelo; γ-terpinene (11.07 %), p-cymene (11.24 %), sabinene (3.30 %), citronellol (1.41 %), Terpinen-4-ol (1.19 %) in rough lemon; linalool (9.14 %), myrcene (1.69 %) in Sikkim mandarin.
- Some of the components which are found in in 800-1000 m but absent in rest are acetophenone, o-Cymen-5-ol, δ-cadinene (ripened stage) and γ-Terpineol acetate, 1 -Nonanal, Trans-Carveol, 2,6-Octadien-1-ol, δ-Cadinene, undecanal (mature green stage) in Sikkim mandarin.
- Likewise in pommelo components like α-Terpinolene, Bicyclo[3.3.0]oct-2-en-7-one, Trans-Chrysanthenyl acetate, o-Cymen-5-ol, Tricyclo [3.1.0.0(2,4)]Hexane, Bicyclo[3.1.1]heptane,Limonen-10-yl-acetate,β-Caryophyllene ,Trans-α-Bergamotene, β -Bisabolene (ripened stage) and Nerol, trans-p-Mentha-2,8-dienol, Cis-p-Mentha-2,8-dien-1-ol, Benzene, 1-ethyl-2,3-dimethyl, nerolidol, (2R,4R)-p-Mentha-6,8-diene, 2-hydroperoxide(mature green stage) were found in 800-1000 m altitude but not in other altitude.
- Rough lemon showed components like Hexadec-(9Z)-enal, Isopulegol 2, Carvacryl methyl ether, o-Cymen-5-ol, Neo isothujol R)-Lavundulyl acetate , Caryophyllene <(E)->, α-Curcumene α-Funebrene, β-Bisabolene ,β-sesquiphellandrene, ar-Turmerol, Isospathulenol, ar- Tumerone α-Tumerone, curlone, 3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl) cyclohex-1-ene, (+)-

Nerolidol, 5-isopropyl-6,6-dimethyl-hept-3-ene-2,5-diol , Carvotanacetone <8-hydroxy->, 2-cyclohexen-1-one, 10-Methyl-8-tetradecen-1-ol acetate, 2-Bornanol , p-Menthane-1,2,4-triol, 2Methylisoborneol, 2-Hydroxy-1,8 cineole, B Citronellal <hydroxy>,2-Furanmethanol (ripened stage) and Cyclo octanol, Menthane-1,2,4-triol, 1,3-Benzenediol ,Cis-p-Mentha-2,8-dien-1-ol, β-Farnesene, 1,4,4-Trimethyl - 2-Cyclohexen-1-yl, Cyclopentan-1,2-dione, (-)-trans-Isopiperitenol ,Cis-Carveol ,α-Limonene diepoxide, Limonene dioxide, p-Mentha-2,8-diene, 1-hydroperoxide,allo-ocimenol , Nerolidol, Nerolidyl acetate ,Trans-α-Bergamotene (mature green stage) were absent in higher altitude and were present only in 800-1000 m altitude.

- Several compounds like α-Thujene, α-Pinene, myrcene, sabinene, octanal, β-ocimene, γ-Terpinene, Terpinen-4-ol, α-Terpineol, linalool decreased in quantity with increasing altitude whereas constituents like camphene, sabinene, limonene, β-pinene increase in quantity with decreasing altitude in ripened and mature green stage of Sikkim mandarin .Moreover in pommelo α-Thujene, myrcene, limonene, β-ocimene, γ-Terpinene compounds tend to decrease and sabinene, α-Pinene, linalool, Terpinen-4-ol, α-Terpineol were found with increase at higher altitude (>1600 m).
- On the other side, rough lemon showed α-Pinene, α-thujene, sabinene, myrcene, γ -Terpinene, linalool, nonanal tend to decrease and that of compounds like β-pinene, octanal, limonene increases with increase in altitude.
- Pectin analysis resulted into sugar and organic acid, % yield on dry basis, alkalinity as carbonate, ash content being leading by pommelo. As that in methoxyl content and AUA were occupied by rough lemon. Further Sikkim

mandarin showed % yield on wet basis, equivalent weight, AUA content DE with the maximum value.

- Pectin extracted from residual peel of different germplasm showed the quality of commercial grade with yield up to 34.67 % of dry peel residue; methoxyl content (7.31 %), DE (90.94 %), ash content (8.83 %)
- Pectin was subjected for FTIR analysis. Absorption band observed at 3618 cm-1 at O-H bond , C-H stretch at 3098 cm-1, C-O bond at 1219 cm-1, C=O bond at 1734 cm-1, C-N stretch (1990 cm-1), C-H wag (1187 cm-1) confirms the structural purity of pectin among the different citrus germplasm.
- The band spectra were similar for pectin extracted from peel of the samples collected from different altitude and maturity stages.
- Ionome profiling was done using Inductively Coupled Plasma Mass Spectrometry (ICPMS) (Perkin Elmer, Nex ION 300 X) system with cross flow nebulizer in view of understanding the nutritive potential of peel and pomace as animal feed. Both in peel and pomace was found to have abundance of major and minor element as N> K> Mg > P> Ca> Na> Cu> S> Fe> Bo> Mo> Zn> Li> Co> Mn.
- On comparison of major elements with commercial feed components like corn cob, wheat, sorghum, maize cob, rice it can be concluded that peel and pomace of these three germplasm can be a potential component of any animal feed.
- Sikkim mandarin and pommelo pomace showed highest potassium content. wherease magnesium content was highest in pommelo peel and pomace which has advantage for those people who are prone to high blood pressure and asthma.

- The iron content was highest in Sikkim mandarin peel which can be recommended for fighting against anaemia, oxidative stress.
- Rough lemon was found with higher calcium content both in peel and pomace and hence can be used as a source of calcium for adults.
- Likewise, zinc was also being highest in pommelo peel and pomace and has advantage in people suffering from osteoporosis, arthritis, night blindness. Moreover, phosphorus and manganese (pommelo pomace), molybdenum and copper (pommelo peel) and hence has advantage for anaemia, muscle weakness and aging problem. Besides that, other trace elements were also found in peel (silver, lithium and cerium) and pomace (sulphur, lead, mercury, nickel and uranium) of pommelo.
- Further, rough lemon peel (sodium, magnesium, boron and barium) and pomace (calcium, aluminium, caesium, silicon and iodine) were recorded as the maximum content.
- On the other hand, peel of Sikkim mandarin was found with nitrogen, phosphorus, calcium, iron, cadmium, mercury, nickel and uranium as the highest and that in pomace was nitrogen, iron, molybdenum, silver, cobalt, lithium, sodium, barium and cerium.

Future line of work

- Essential oil can be assessed for antimicrobial activities which may in turn be used as a biopesticides.
- Value addition and product development using essential oils of citrus peel.
- Use of citrus peel and pomace in feed industry and food fortification.
- Explore the possibility of using citrus peel for commercial pectin production.
- Explore the possibility on commercializing pommelo and rough lemon.

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Gangtok, 2020 **(Anjana Pradhan)**

Fruit
Peel

Cross section of fruit

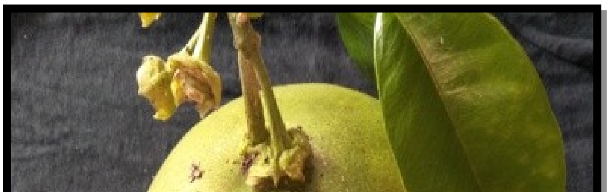
Pomace

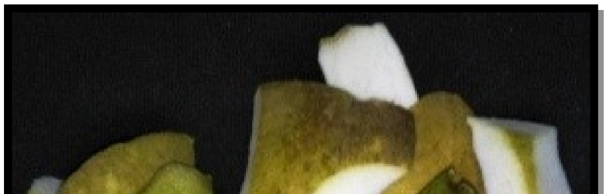
Plate No 1: Sikkim mandarin (*Citrus reticulata* L) in mature green stage

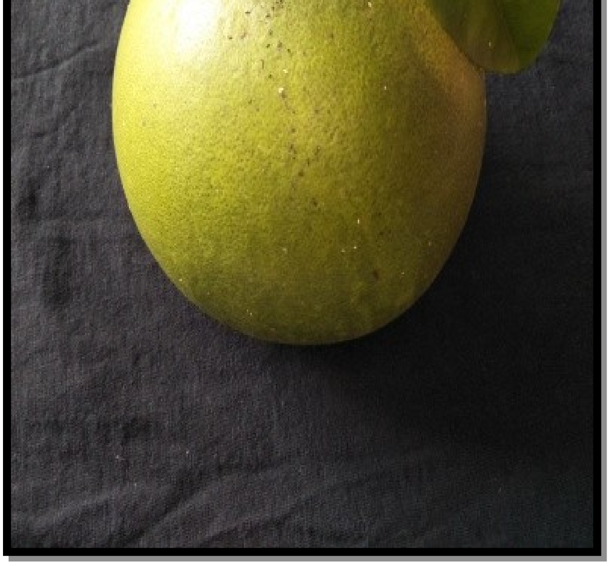
Fruit **Peel**

Cross section of fruit **Pomace**

Plate No 2: Sikkim mandarin (*Citrus reticulata* L) in ripened stage







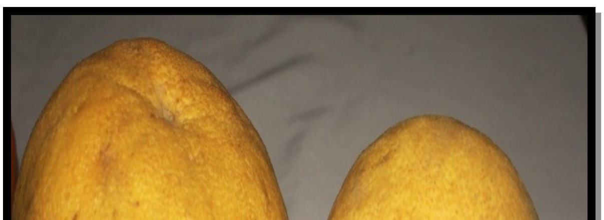
Fruit

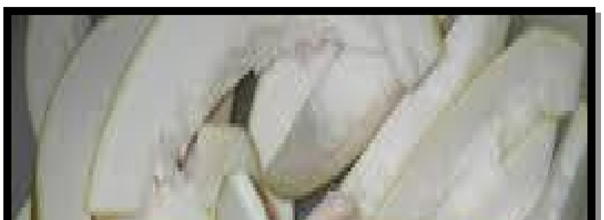
Peel

Cross section of fruit

Pomace

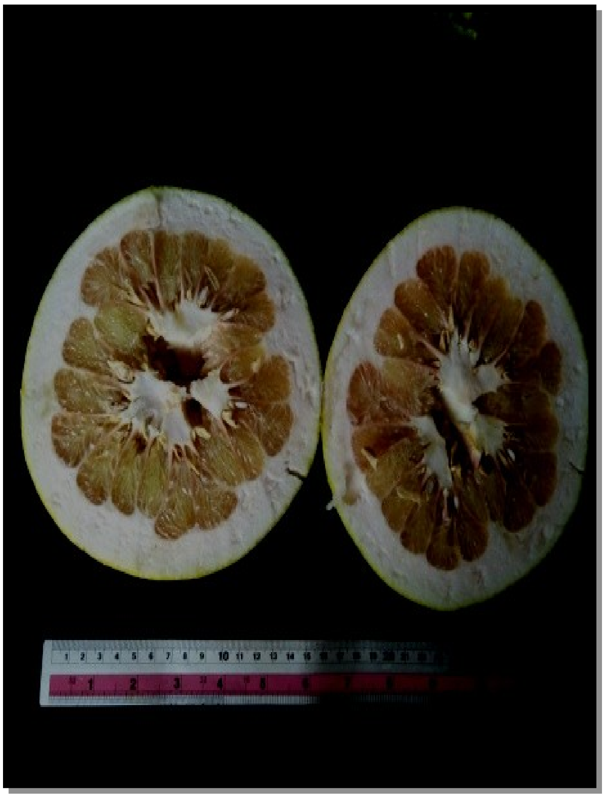
Plate No 3: Pommelo (*Citrus maxima* Merr.) in mature green stage





Fruit

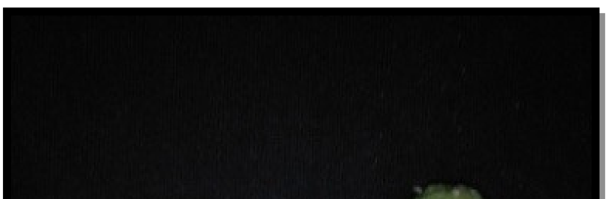
Peel

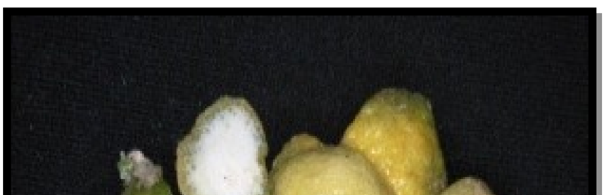


Cross section of fruit

Pomace

Plate No 4: Pommelo (*Citrus maxima* Merr.) in ripened stage

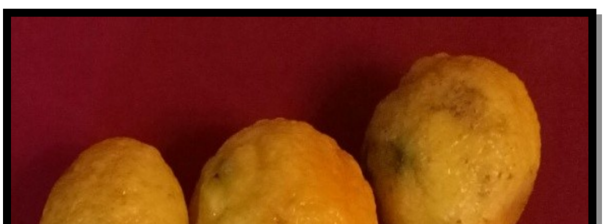


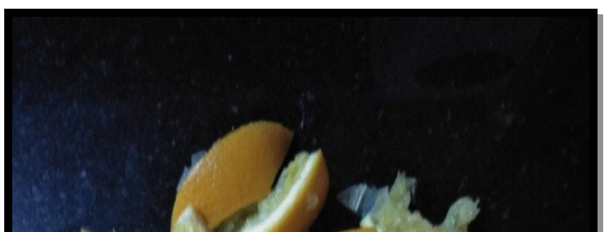


Fruit **Peel**

Cross section of fruit **Pomace**

Plate No 5: Rough lemon (*Citrus jambhiri* Lush) in mature green stage





Fruit

Peel

Cross section of fruit

Pomace

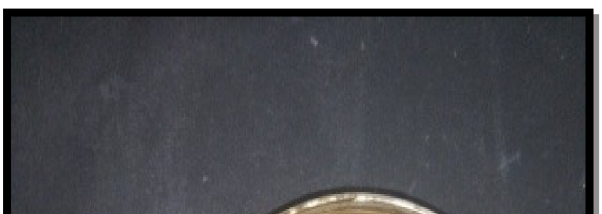
Plate No 6 : Rough lemon (*Citrus jambhiri* Lush) in ripened stage

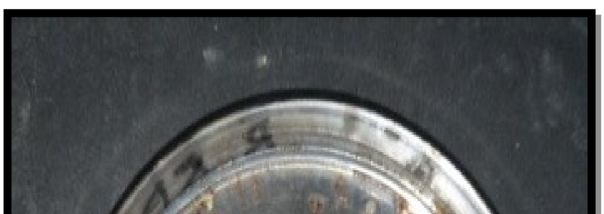
Dried peel of Citrus fruit

Jelly pectin liberated

Boiling of peel with citric acid solution

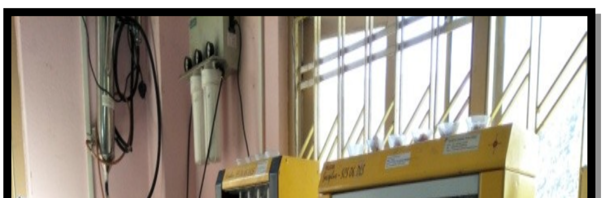
Fumes appear during boiling





Jelly pectin after filter **Jelly pectin dried up**

Plate No. 7: Steps in pectin extraction



Crude fat estimation

Specific gravity determination of essential oil

Preparation of sample for open air digestion

Refractive index determination of essential oil

Sample preparation for characterization of essential oil

Weighing sample

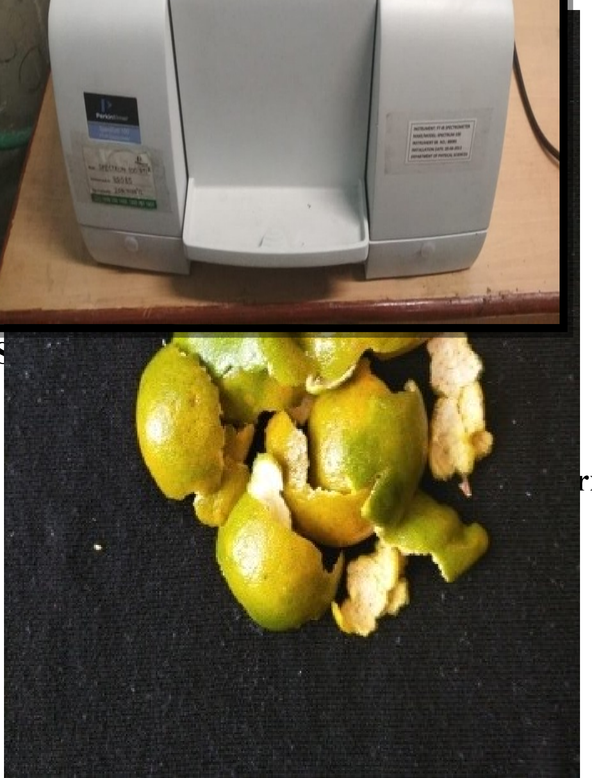
Plate No. 8: Experiment performed in Lab

GCMS analysis of essential oil

UV vis spectrophotometer for antioxidant activity

S

ICPMS analysi of peel and pomace



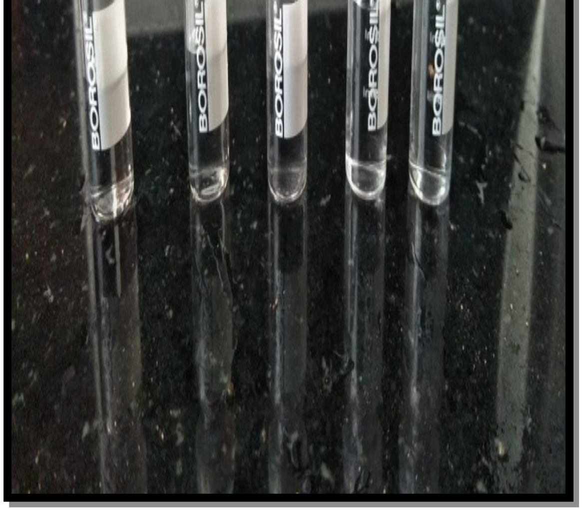
rmed in different instruments

Peel of fruit (sample)

Hydro distillation by Clevenger apparatus

Loaded peel in round bottom flask

Extraction process



Oil liberated **Essential oil in vials**

Plate No 10. Extraction of essential oil by Clevenger apparatus

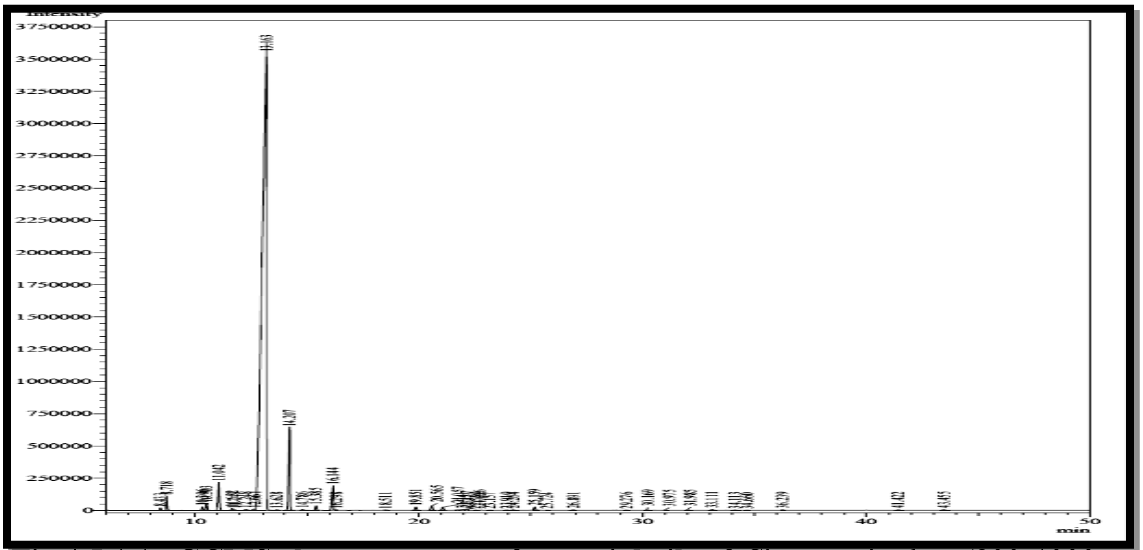


Fig.4.5.1.1a GCMS chromatogram of essential oils of *Citrus reticulata* (800-1000 m altitude; mature green stage)

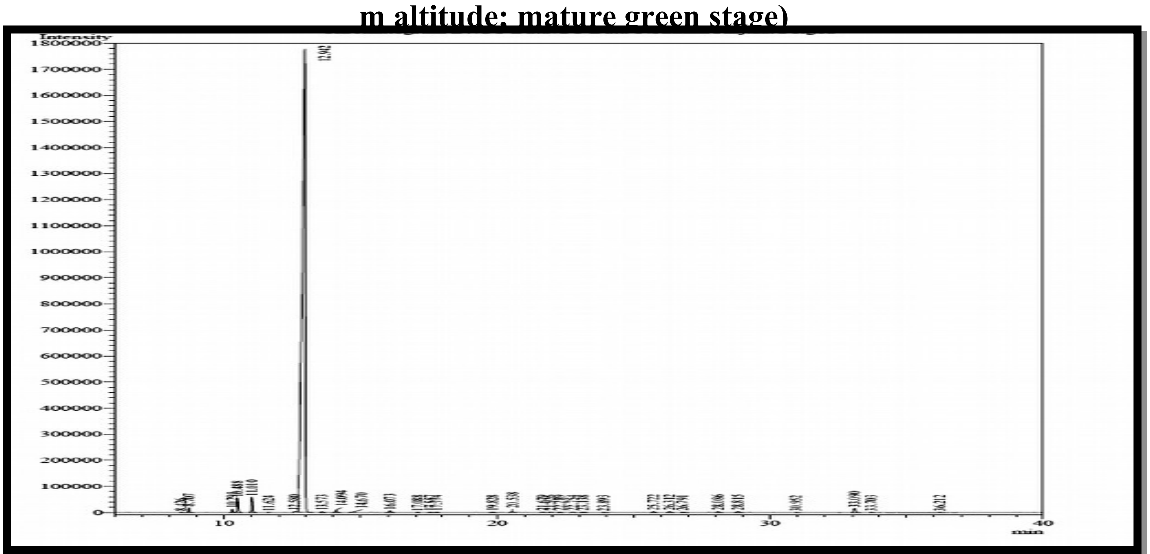


Fig.4.5.1.1b GCMS chromatogram of essential oils at *Citrus maxima* (800-1000 m altitude; mature green stage)

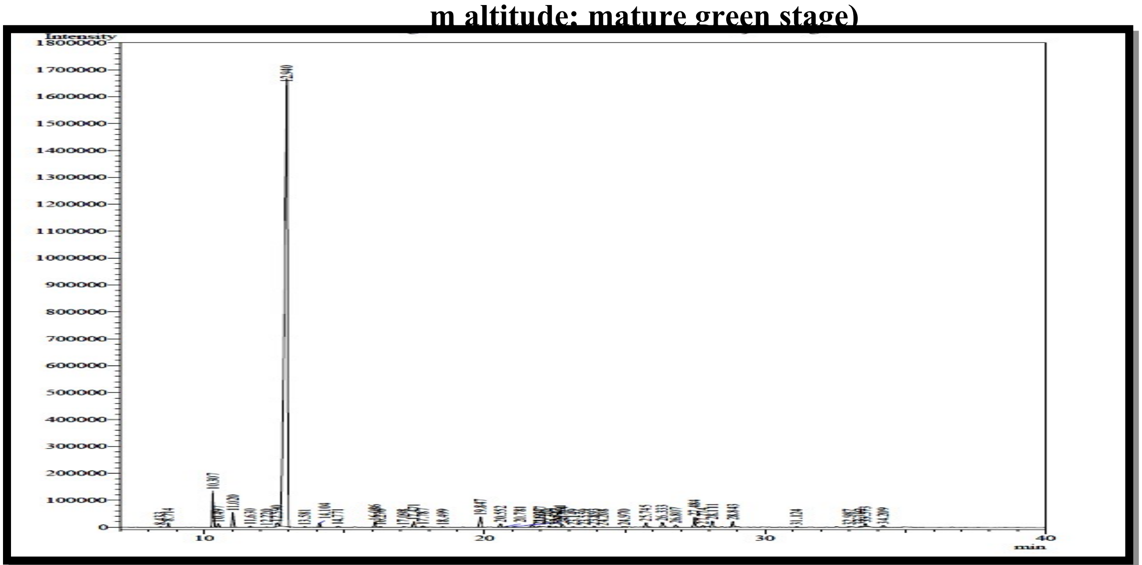


Fig.4.5.1.1c GCMS chromatogram of essential oils of *Citrus jambhiri* (800-1000 m altitude ; mature green stage)

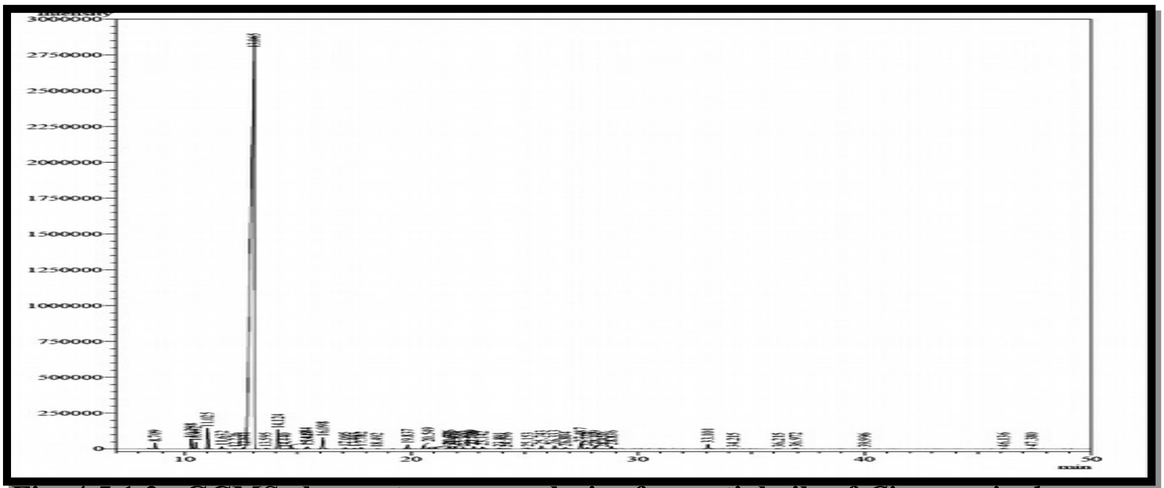


Fig. 4.5.1.2a GCMS chromatogram analysis of essential oils of *Citrus reticulata* (1000-1200 m altitude; mature green stage)

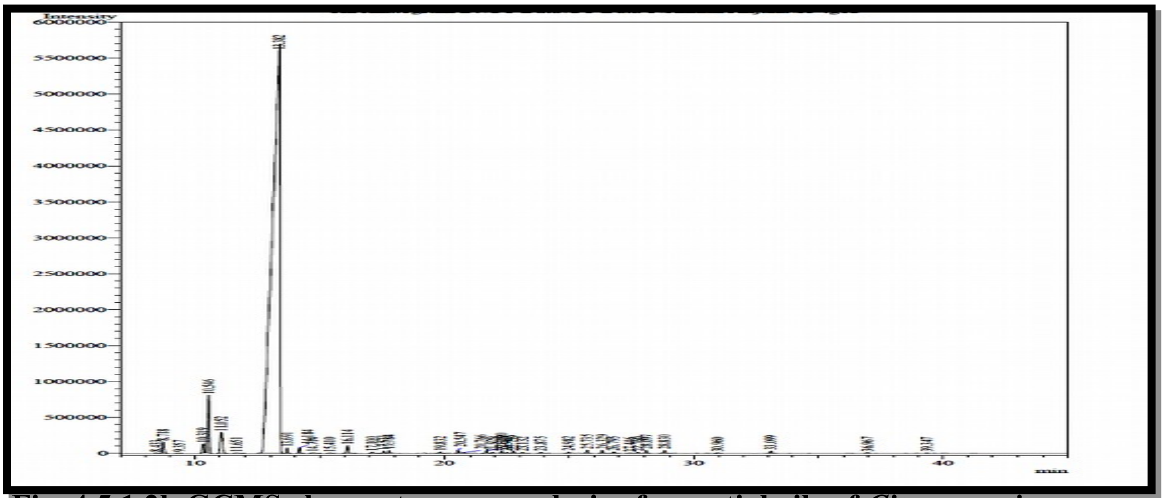


Fig. 4.5.1.2b GCMS chromatogram analysis of essential oils of *Citrus maxima* (1000-1200 m altitude; mature green stage)

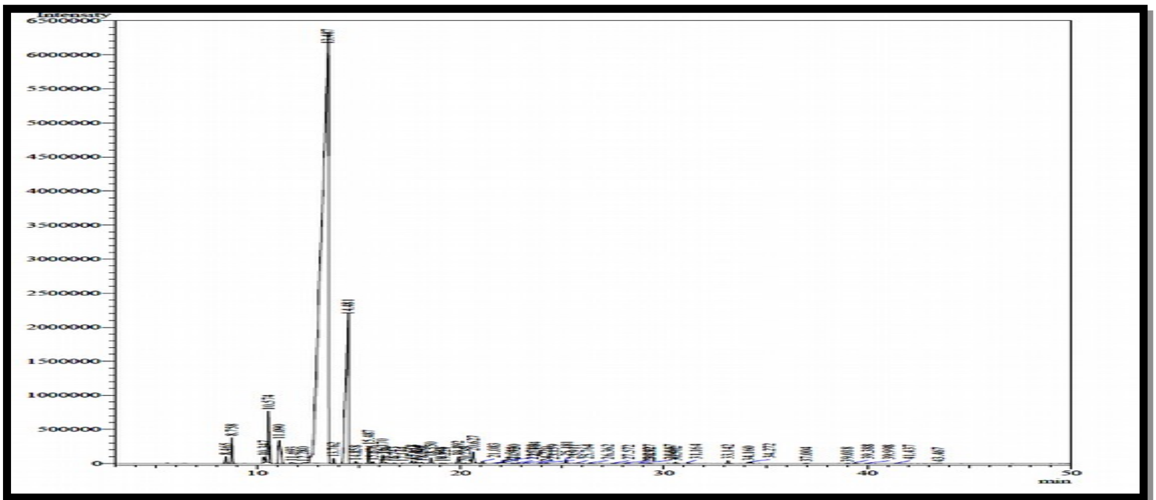


Fig. 4.5.1.2c. GCMS chromatogram analysis of essential oils of *Citrus jambhiri* at (1000-1200 m altitude ;mature green stage)

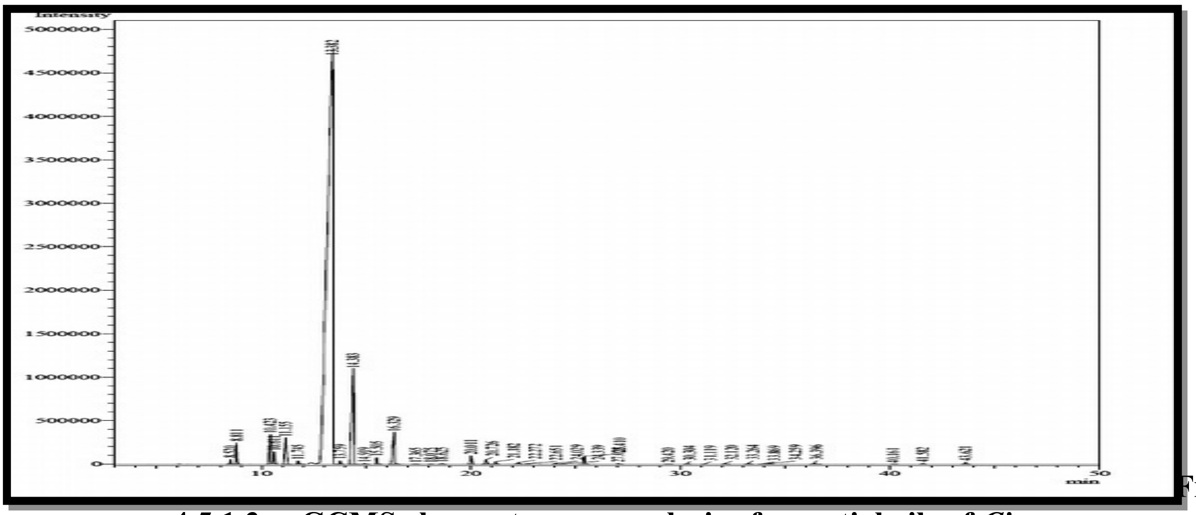


Fig.4.5.1.3 a. GCMS chromatogram analysis of essential oils of *Citrus reticulata* (1200-1400 m altitude; mature green stage)

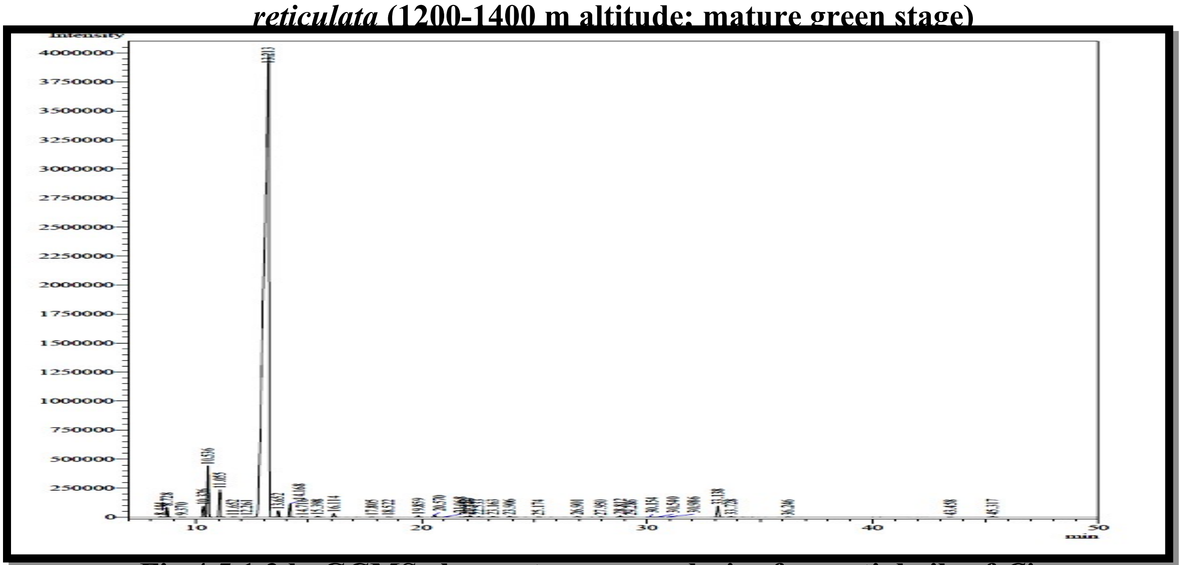


Fig.4.5.1.3 b. GCMS chromatogram analysis of essential oils of *Citrus maxima* (1200-1400 m altitude; mature green stage)

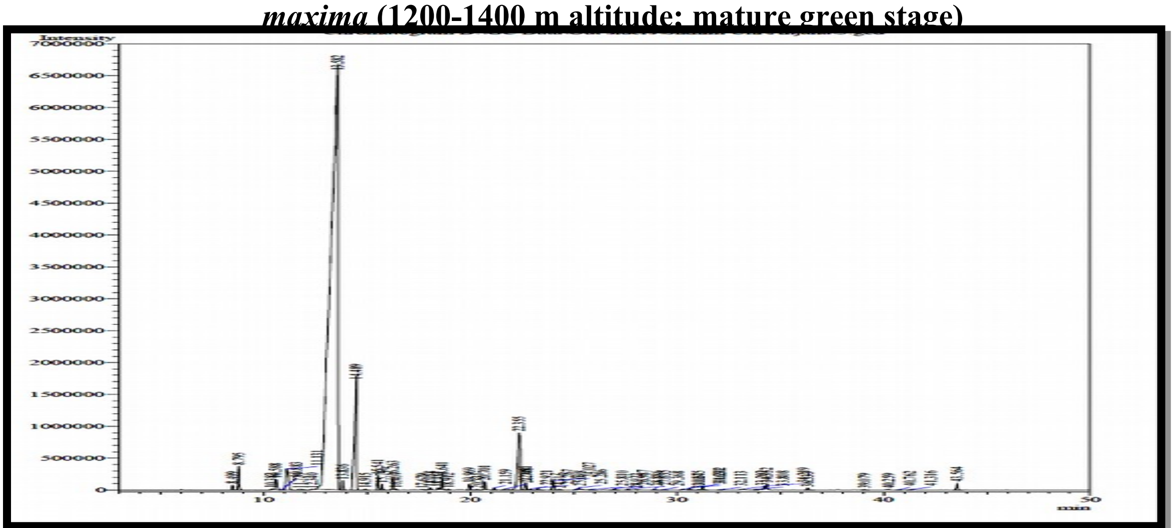


Fig.4.5.1.3 c. GCMS chromatogram analysis of essential oils of *Citrus jambhiri* (1200-1400 m altitude; mature green stage)

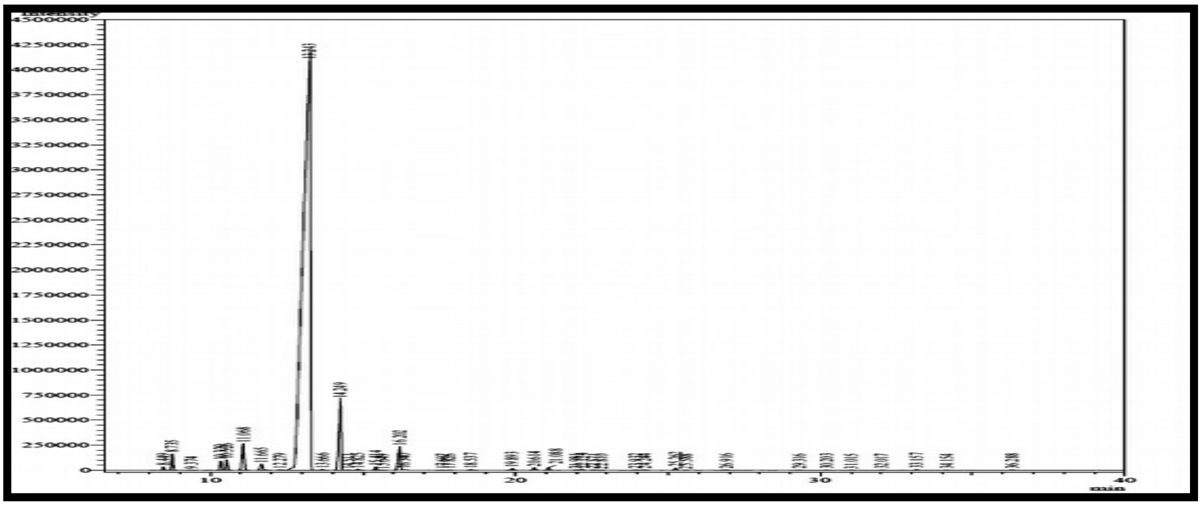


Fig. 4.5.1.4 a. GCMS chromatogram analysis of essential oils of *Citrus reticulata* (1400-1600 m altitude; mature green stage)

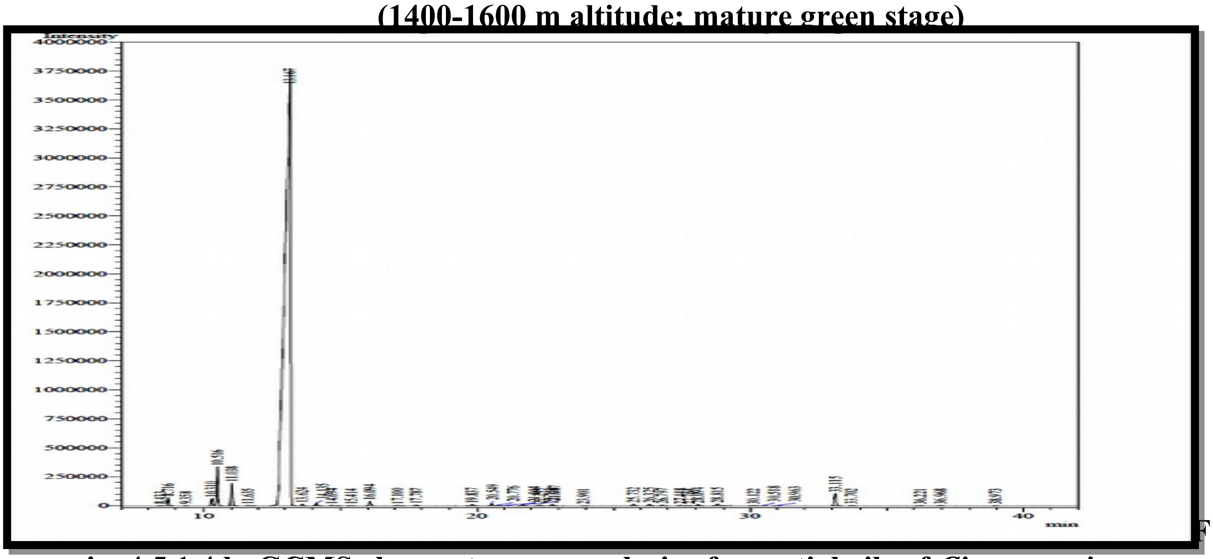


Fig. 4.5.1.4 b. GCMS chromatogram analysis of essential oils of *Citrus maxima* (1400-1600 m altitude; mature green stage)

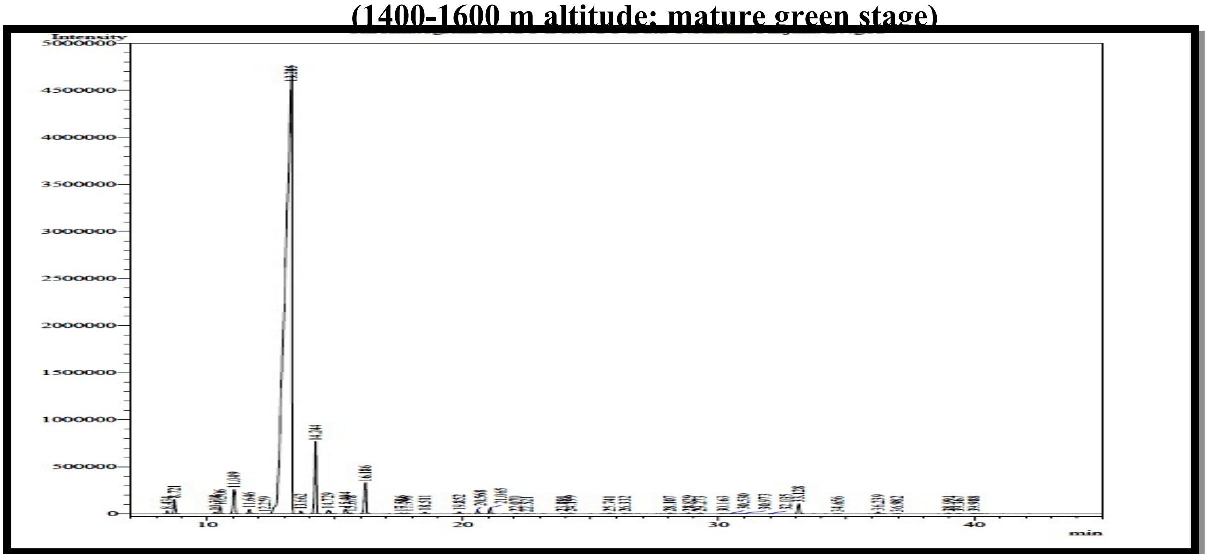


Fig. 4.5.1.4 b. GCMS chromatogram analysis of essential oils of *Citrus jambhiri* (1400-1600 m altitude; mature green stage)

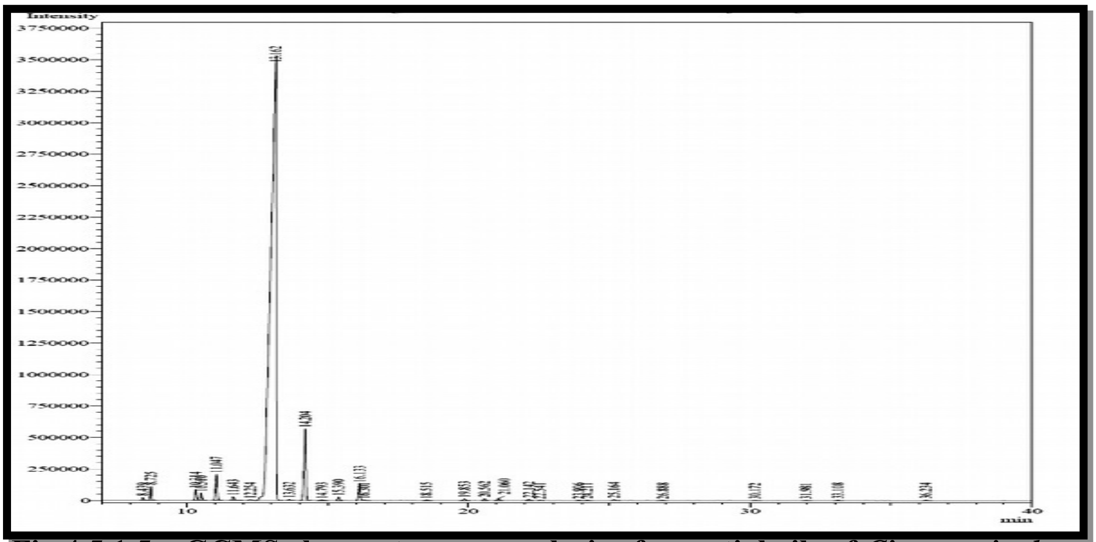


Fig.4.5.1.5a. GCMS chromatogram analysis of essential oils of *Citrus reticulata* (>1600 m altitude; mature green stage)

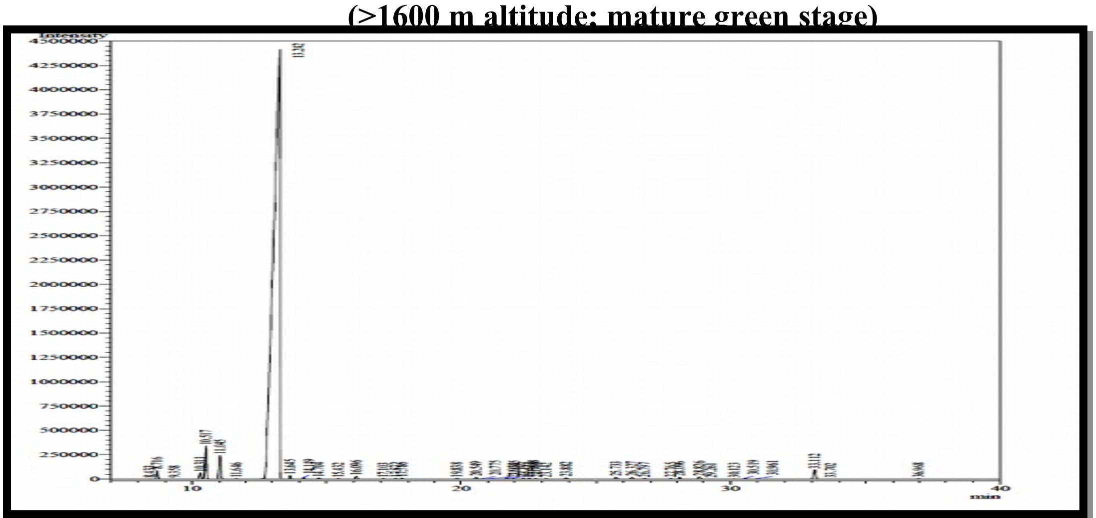


Fig.4.5.1.5a. GCMS chromatogram analysis of essential oils of *Citrus maxima* (>1600 m altitude; mature green stage)

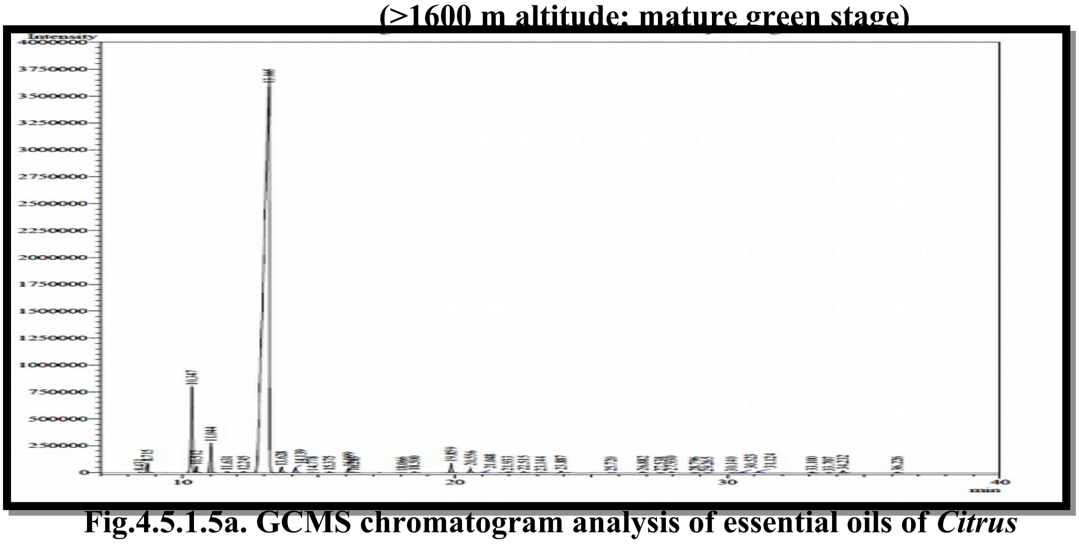


Fig.4.5.1.5a. GCMS chromatogram analysis of essential oils of *Citrus jambhiri* (>1600 m altitude ;mature green stage)

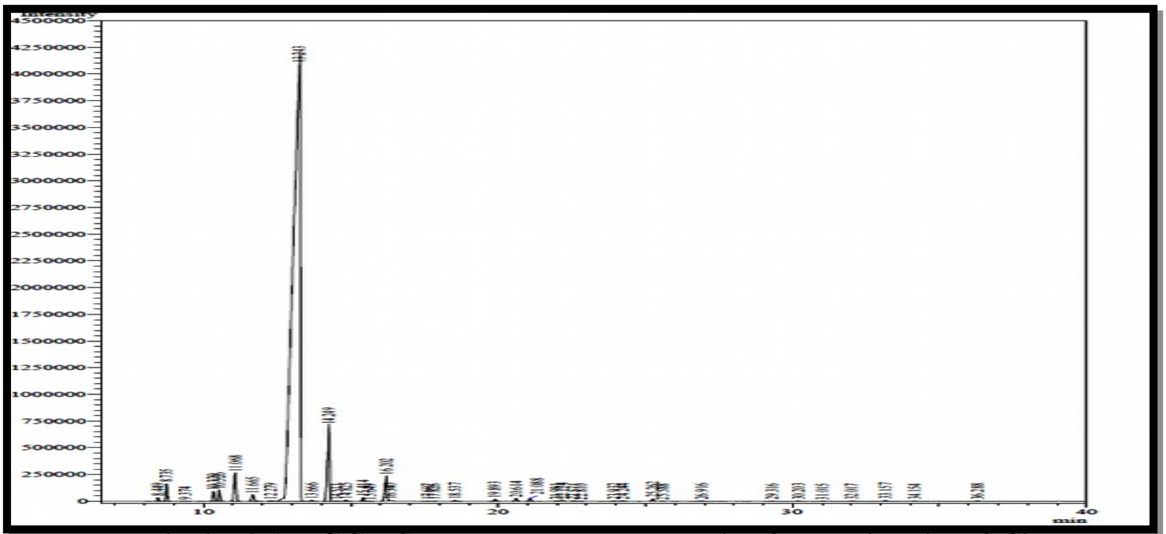


Fig.4.5.2.1a. GCMS chromatogram analysis of essential oils of *Citrus reticulata* (800-1000 m altitude ;ripened stage)

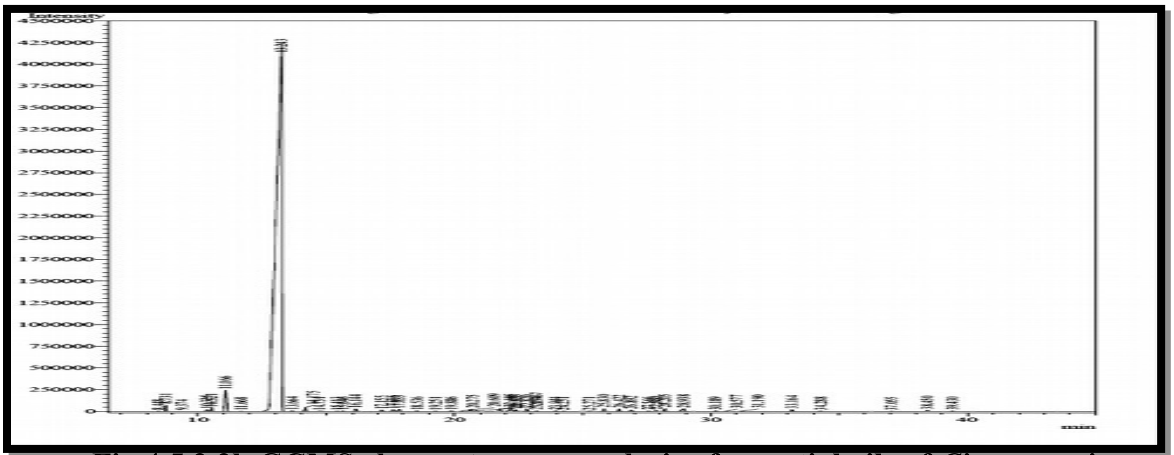


Fig 4.5.2.2b GCMS chromatogram analysis of essential oils of *Citrus maxima* (800-1000 m altitude ;ripened stage)

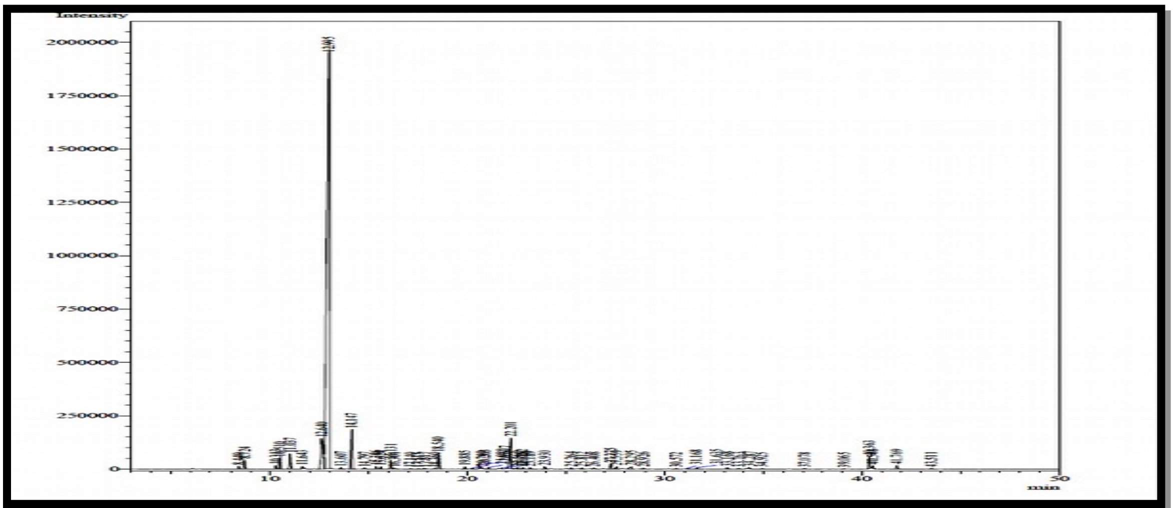


Fig 4.5.2.3cGCMS chromatogram analysis of essential oils of *Citrus jambhiri* (800-1000 m altitude ;ripened stage)

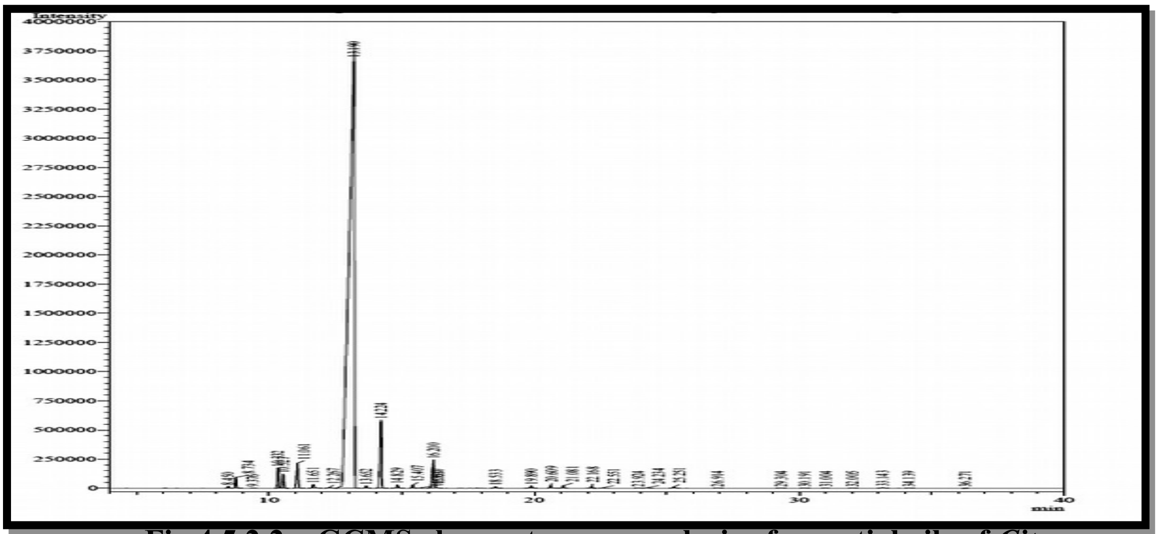


Fig 4.5.2.2 a GCMS chromatogram analysis of essential oils of *Citrus reticulata* (1000-1200 m altitude ;ripened stage)

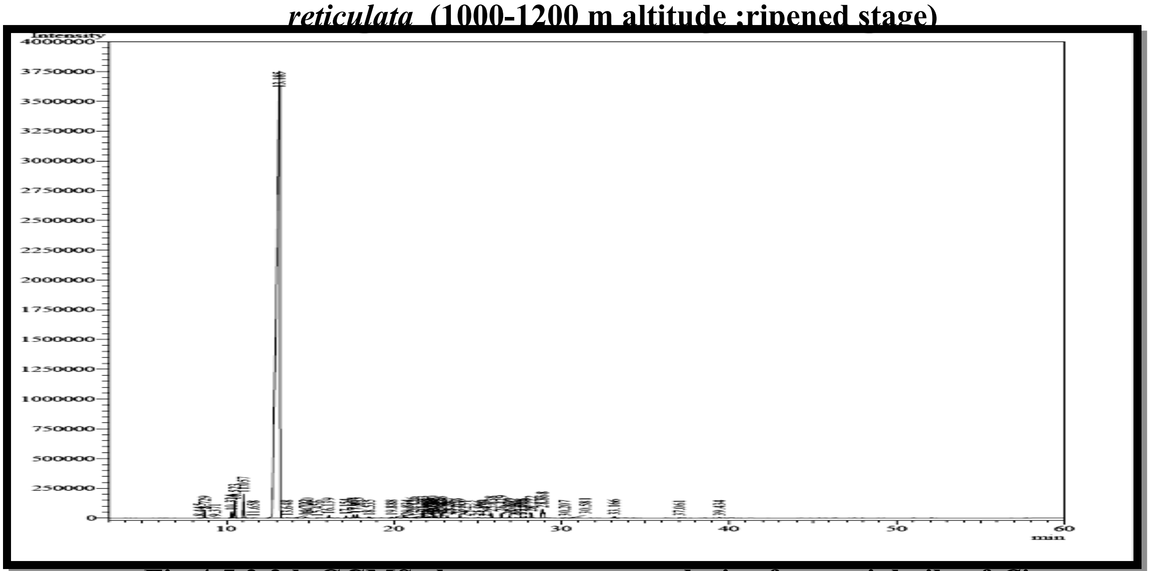


Fig 4.5.2.2 b GCMS chromatogram analysis of essential oils of *Citrus maxima* (1000-1200 m altitude ; ripened stage)

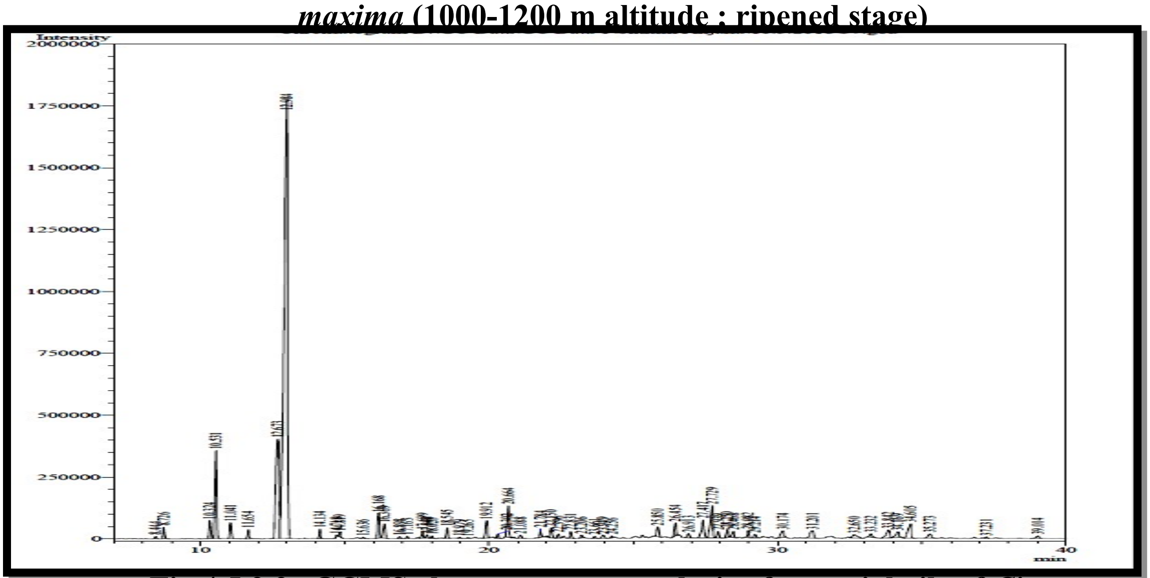


Fig 4.5.2.2c GCMS chromatogram analysis of essential oils of *Citrus jambhiri* (1000-1200 m altitude ; ripened stage)

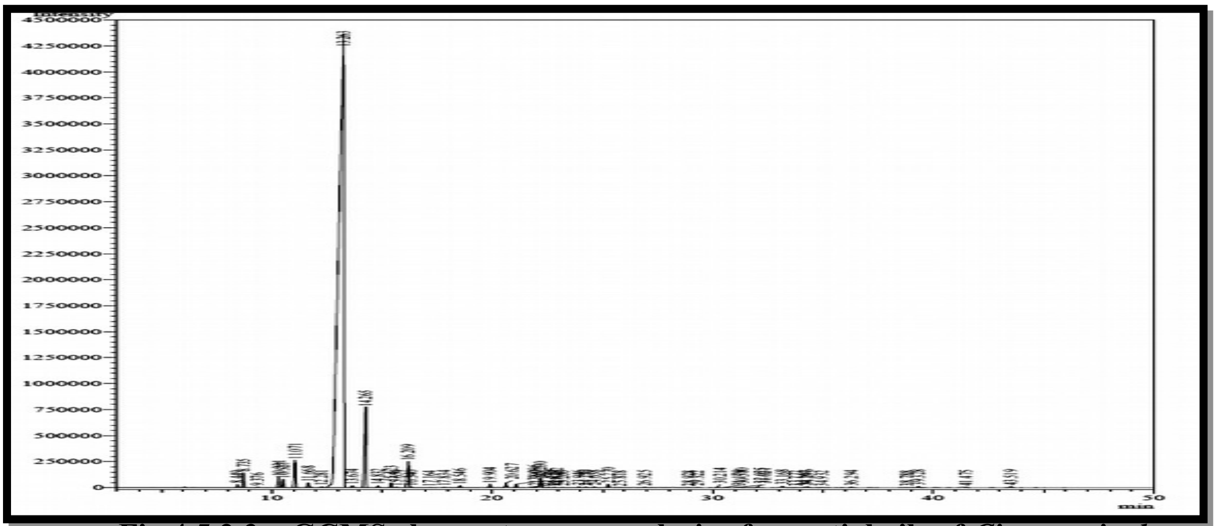


Fig 4.5.2.3.a GCMS chromatogram analysis of essential oils of *Citrus reticulata* (1200-1400 m altitude ; ripened stage)

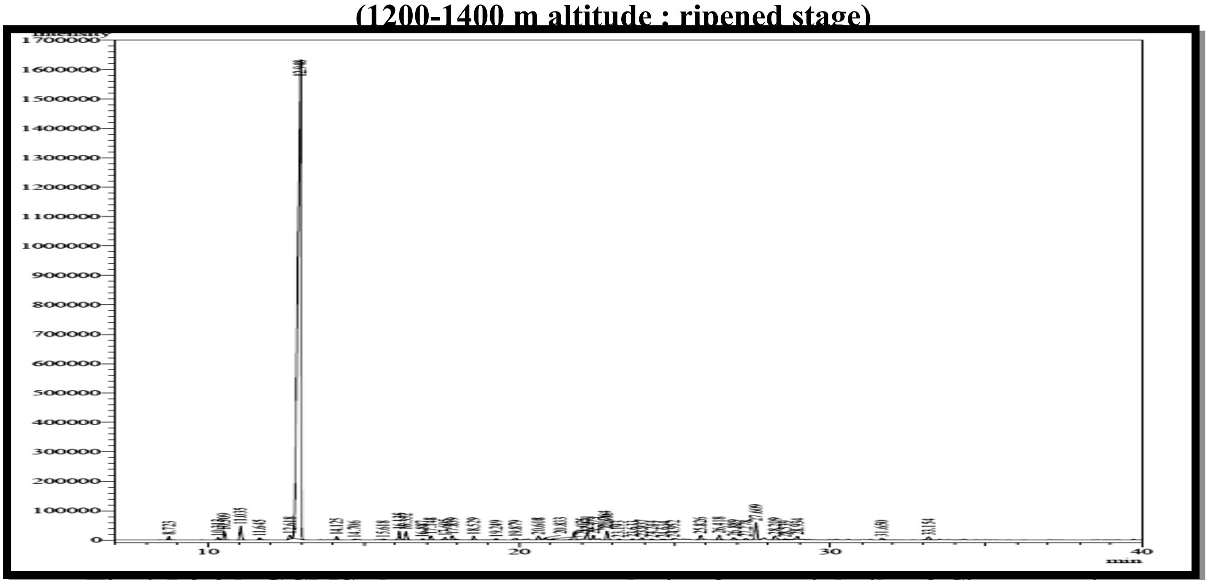


Fig 4.5.2.3.b GCMS chromatogram analysis of essential oils of *Citrus maxima* (1200-1400 m altitude ; ripened stage)

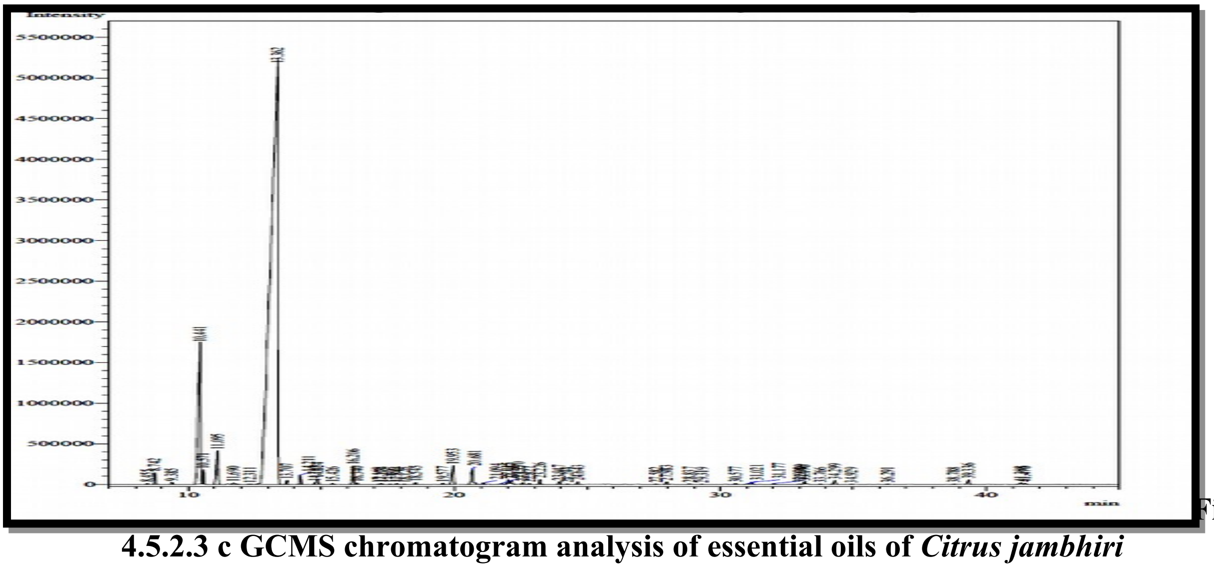


Fig 4.5.2.3 c GCMS chromatogram analysis of essential oils of *Citrus jambhiri* (1200-1400 m altitude ; ripened stage)

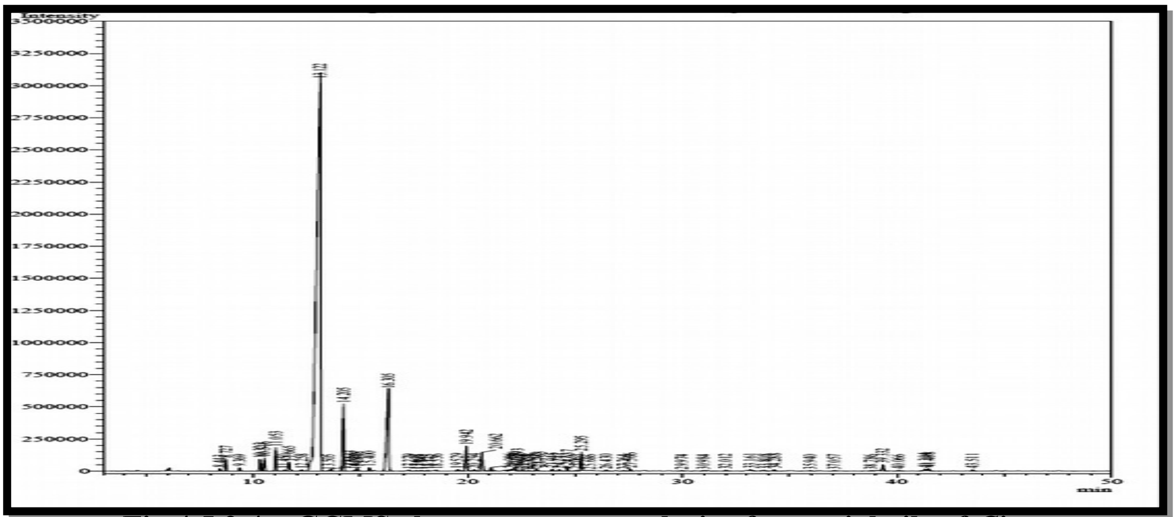


Fig 4.5.2.4 a GCMS chromatogram analysis of essential oils of *Citrus reticulata* (1400-1600 m altitude ; ripened stage)

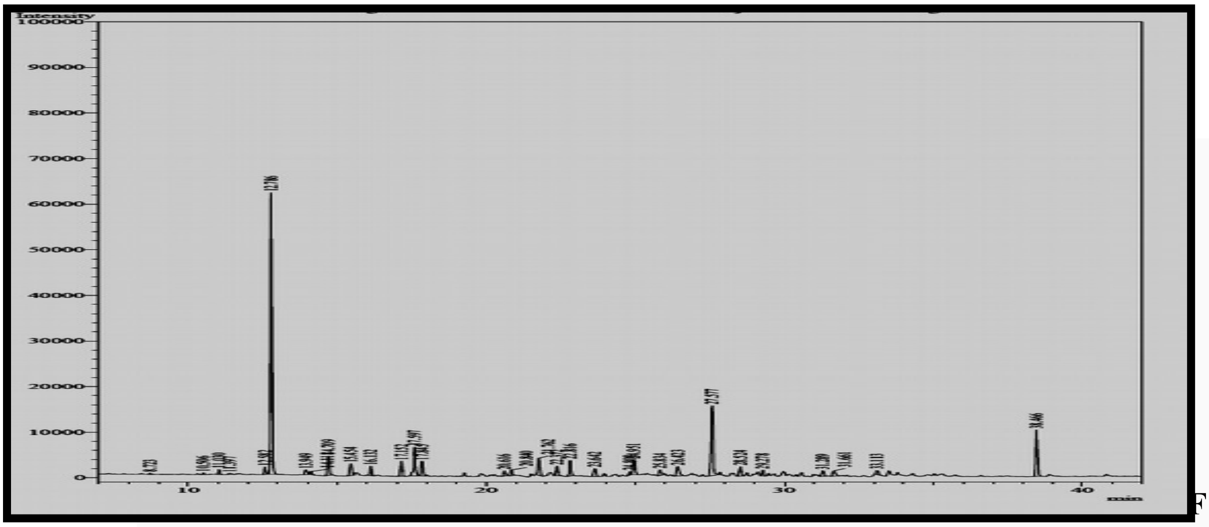


Fig 4.5.2.4 b GCMS chromatogram analysis of essential oils of *Citrus maxima* (1400-1600 m altitude; ripened stage)

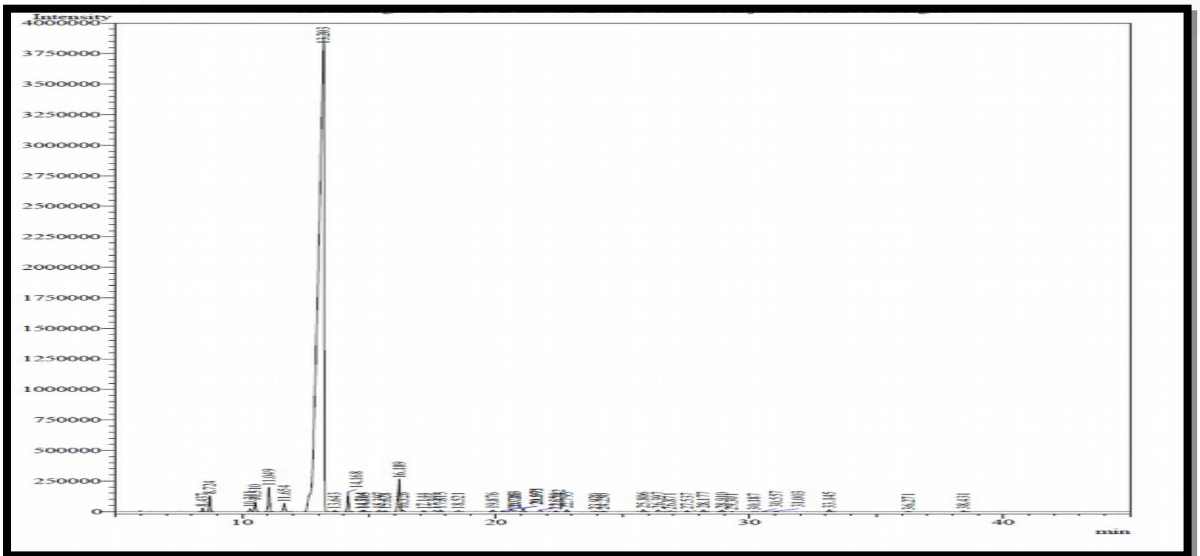


Fig 4.5.2.4 c GCMS chromatogram analysis of essential oils of *Citrus jambhiri* (1400-1600 m altitude; ripened stage)

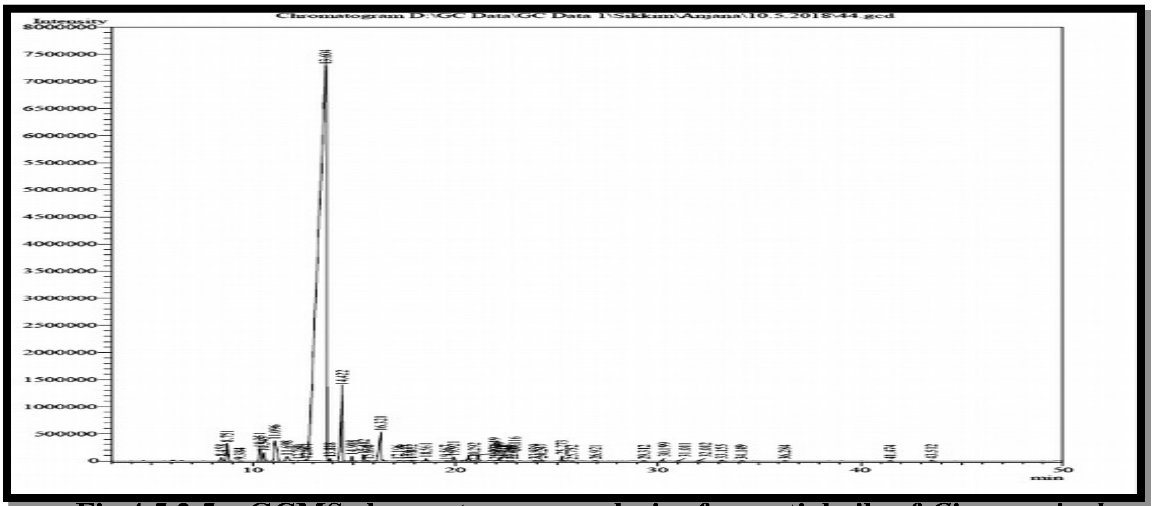


Fig 4.5.2.5 a GCMS chromatogram analysis of essential oils of *Citrus reticulata*
(>1600 m atitude ;ripened stage)

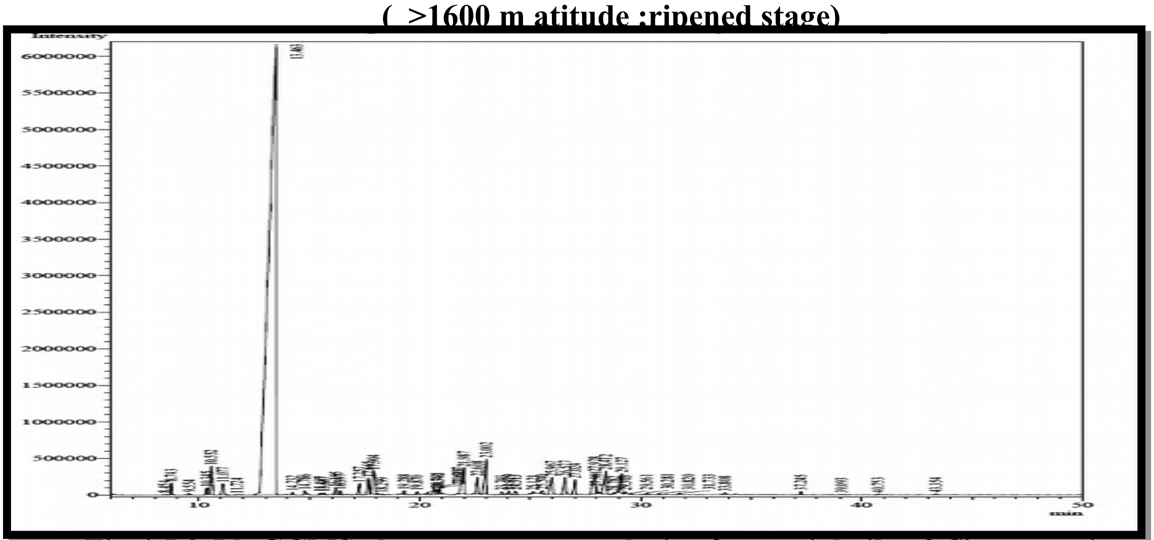


Fig 4.5.2.5 b GCMS chromatogram analysis of essential oils of *Citrus maxima*
(>1600 m altitude : ripened stage)

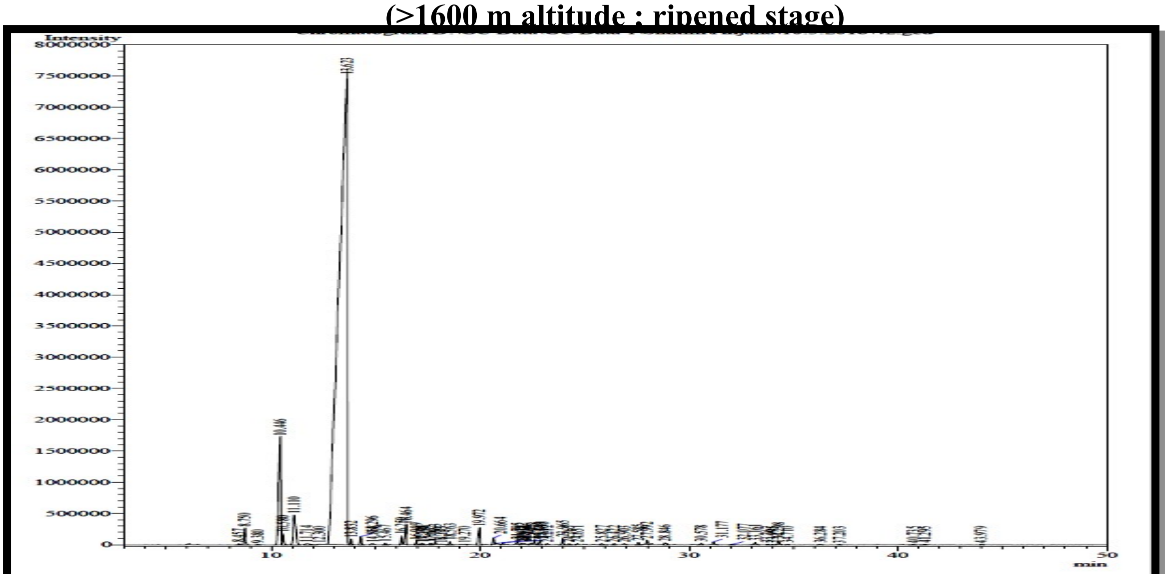


Fig 4.5.2.5 c GCMS chromatogram analysis of essential oils of *Citrus jambhiri*
(>1600 m altitude ;ripened stage)

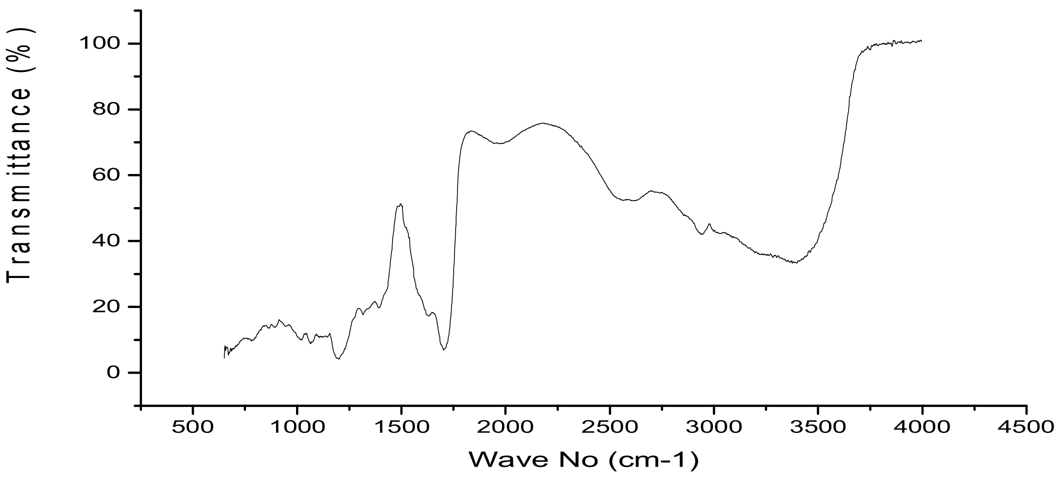


Fig.4.6.2.7 a FTIR spectra of pectin of *Citrus reticulata* at mature green stage

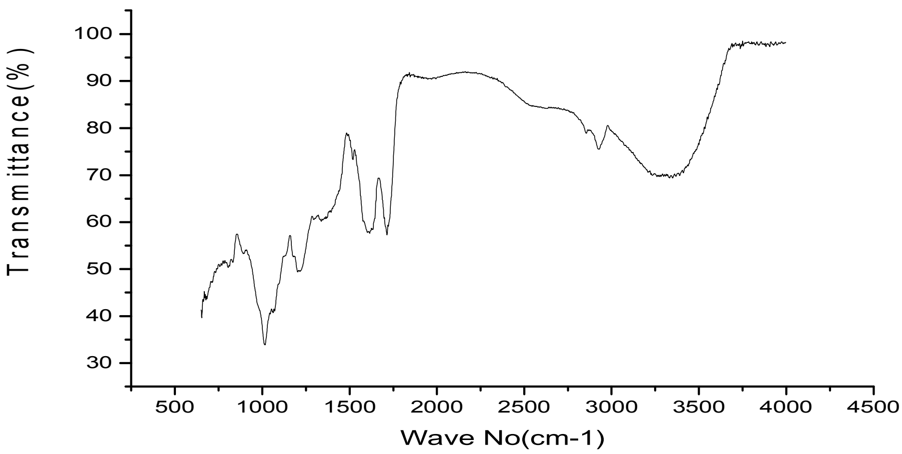


Fig.4.6.2.7b FTIR spectra of pectin of *Citrus maxima* at mature green stage)

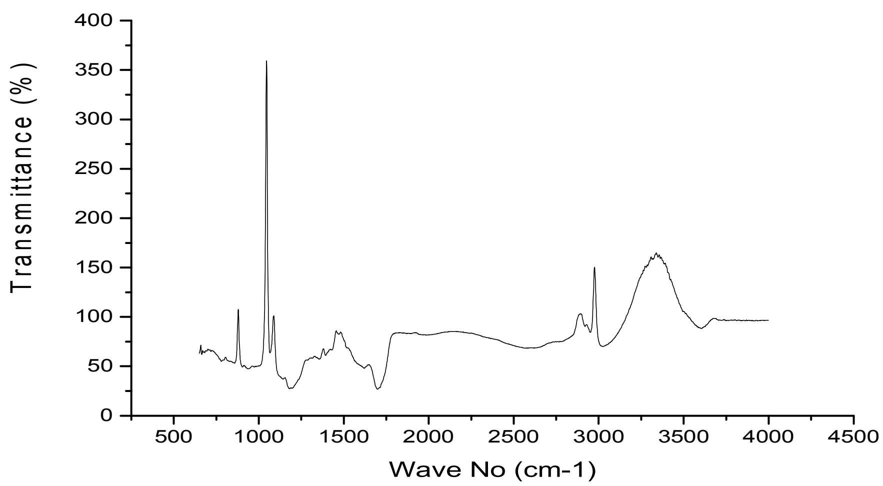


Fig.4.6.2.7c FTIR spectra of pectin of *Citrus jambhiri* at mature green stage)

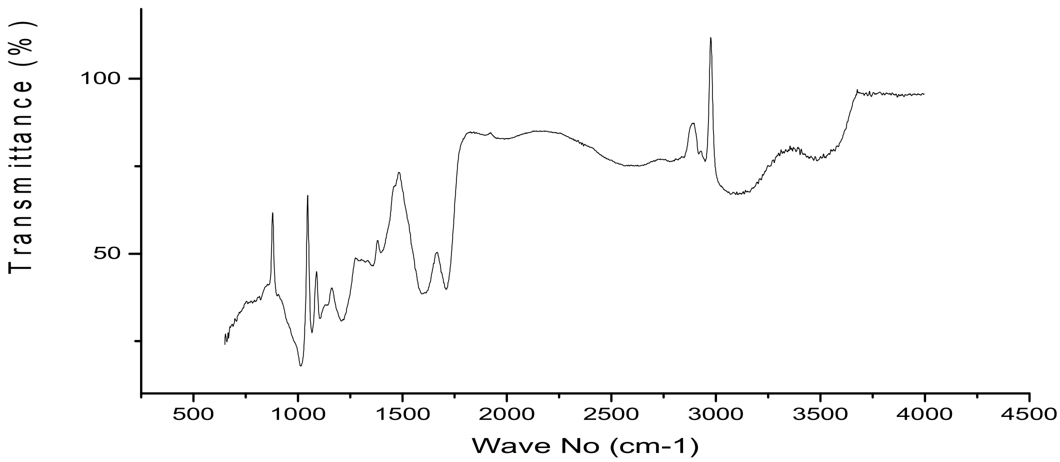


Fig.4.6.2.8 a FTIR spectra of pectin of *Citrus reticulata* at ripened stage

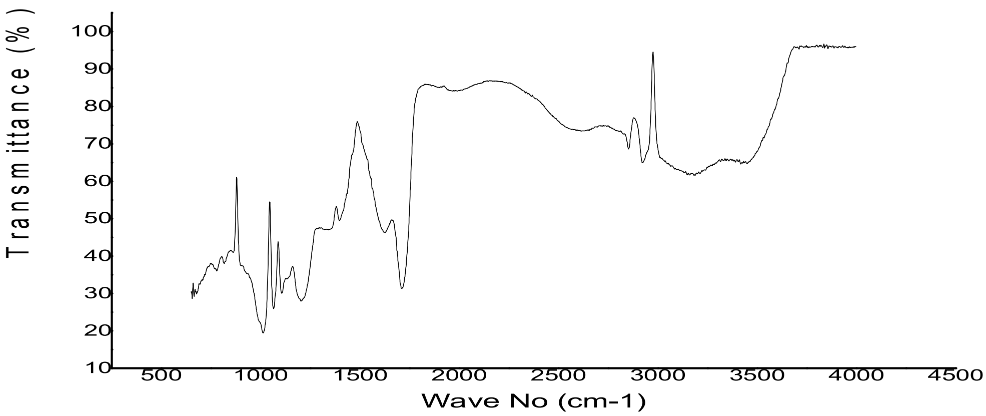


Fig.4.6.2.8 b FTIR spectra of pectin of *Citrus maxima* at ripened stage

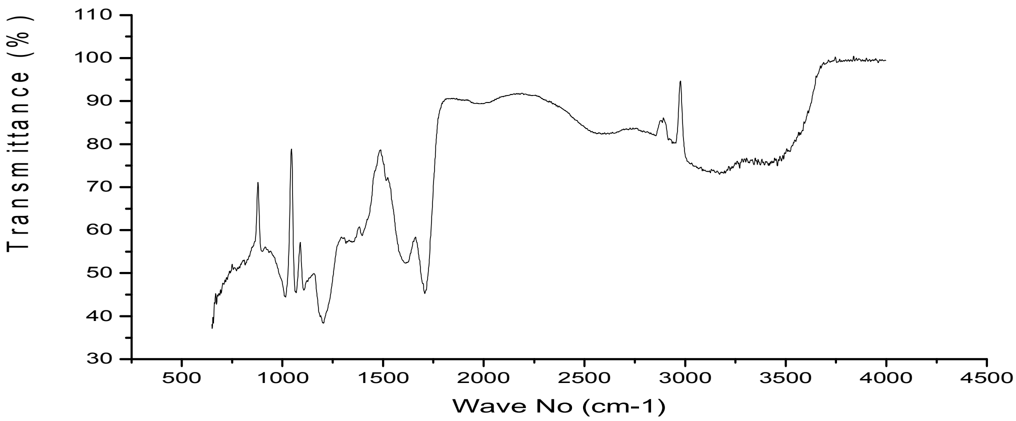


Fig.4.6.2.8c FTIR spectra of pectin of *Citrus jambhiri* at ripened stage

Chapter 7

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CHAPTER 1

INTRODUCTION

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 4

RESULTS

CHAPTER 5

DISCUSSION

CHAPTER 6

SUMMARY AND CONCLUSION

CHAPTER 7

BIBLIOGRAPHY

Declaration

I declare that the present Ph.D. thesis entitled "**Essential oil, pectin and ionome profiling of citrus fruit waste**" submitted by me for the award of the degree of Doctor of Philosophy in Horticulture to the Sikkim University under the supervision of Dr Laxuman Sharma, Associate Professor, Department of Horticulture, Sikkim University is my original research work solely carried out by me in the Department of Horticulture, School of Life sciences, Sikkim University, Gangtok. The thesis has not been submitted for any other degree or diploma in any other university/institution.

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