POST HARVEST CHARACTERISATION AND MANAGEMENT OF AVOCADO (*Persea americana* Mill.)

By

GEETHU M.

(2018-22-006)

DEPARTMENT OF POST HARVEST TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR -680 656

KERALA, INDIA

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DEPARTMENT OF POST HARVEST TECHNOLOGY

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VELLANIKKARA, THRISSUR -680 656

KERALA, INDIA

2022

DECLARATION

I, hereby declare that this thesis entitled **"POST HARVEST CHARACTERISATION AND MANAGEMENT OF AVOCADO (*Persea americana* Mill.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara **Geethu M**

Date: 03.03.2022 (2018-22-006)

CERTIFICATE

Certified that this thesis entitled **"POST HARVEST CHARACTERISATION AND MANAGEMENT OF AVOCADO (*Persea americana* Mill.)"** is a bonafide record of research work done independently by **Ms. Geethu M. (2018-22-006)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Vellanikkara

Date: 03.03.2022

Dr. Saji Gomez

(Chairperson, Advisory Committee)
Associate Professor
Department of Post Harvest Technology
College of Agriculture, Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Geethu M (2018-22-006)**, a candidate for the degree of **Doctor of Philosophy in Horticulture**, with major field in **Post Harvest Technology**, agree that the thesis entitled **"POST HARVEST CHARACTERISATION AND MANAGEMENT OF AVOCADO (*Persea americana* Mill.)"** may be submitted by **Ms. Geethu M.** in partial fulfilment of the requirement for the degree.

3/3/22

Dr. Saji Gomez
Associate Professor
Department of Post Harvest Technology
College of Agriculture, Vellanikkara

3/3/22

Smt. Meagle Joesph P
Associate Professor and Head
Department of Post Harvest Technology
College of Agriculture, Vellanikkara

3/3/2022

Dr. K. Ajith Kumar
Associate Director of Research and Dean
RARS and College of Agriculture,
Ambalavayal, Wayanad

03/03/22

Dr. Jyothi Bhaskar
Professor and Head
Department of Fruit Science
College of Agriculture, Vellanikkara

3/3/2022

Dr. Seeja Thomachan Panjikkaran
Associate Professor and Head
Department of Community Science
College of Agriculture, Vellanikkara

3.3.2022

EXTERNAL EXAMINER
Dr. K.R. VASUDEVA
Professor - PHT
Dept. of Postharvest Technology
College of Horticulture
UHS Campus, Bengaluru - 560 065

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Dedicated to

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LIST OF ABBREVIATIONS

^{o}C	: degree Celsius
µg	: microgram
mg	: milligram
g	: gram
kg	: kilogram
mM	: milli Molar
nm	: nanometre
mm	: millimetre
ml	: millilitre
l	: Litre
ppm	: parts per million
min.	: minutes
h	: hour
%	: percentage
Eq.	: equivalent
et al.	: coworkers
CD	: critical difference
Fig.	: figure
no.	: number
cfu	: colonies forming unit
FW	: Fresh weight
DW	: Dry weight
DM	: Dry matter

INTRODUCTION

1. INTRODUCTION

Avocado (*Persea americana* Mill), is a subtropical fruit belonging to the family Lauraceae, native to Central and South America (Koller, 1992). It is an introduced fruit crop to India from Sri Lanka in the early twentieth century and is grown in a limited area scattered in the south central parts in Tamil Nadu, Kerala, Maharashtra, Karnataka and the eastern Himalayan state of Sikkim. The three races evolved in different climatic environments are Mexican race (*Persea americana var. drymifolia*), evolved in the highlands of south-central Mexico is adapted to tropical highlands (semitropical climate); Guatemalan race, *P. americana var. guatemalensis* is adapted to medium elevations in the tropics, prefers subtropical climate; West Indian (or Antillean) race *P. americana var. americana* is adapted to the lowlands and humid subtropics, grows in tropical areas (Litz *et al*., 2005). All the three races *viz*, West Indian, Guatemalan and Mexican have been adapted to the tropical and sub-tropical conditions in India. In Kerala, avocado is being cultivated mainly in high altitude regions such as Wayanad and Idukki districts and to a very limited extent in plains. Nair and Chandran (2018) mentioned that Pollock, Kallar Round, Purple Hybrid and Fuerte are the major cultivars that are being grown in Kerala. A large number of avocado genotypes are available which vary widely in terms of its biochemical and morphological attributes. Further seed propagation has resulted in considerable variability in biochemical and morphological attributes of fruits, besides tree characteristics (Augustine, 2020).

Avocado fruit has widely varying morphological and physico-chemical properties and nutritional contents, but it is not common and familiar to most parts of Kerala. Natural dispersion of seeds and their subsequent growth into trees have resulted in considerable variability among genotypes found presently in Kerala. Due to the presence of numerous flowers in a smaller area and their peculiar mode of pollination (protogynous diurnally synchronous dichogamy), the avocado tree has wide genetic variability. Therefore, selection of superior nutritional and morphological fruit characters will be of immense significance from the consumer point of view. Studies revealed that there are more than 500 varieties in avocado, but most of them are not acceptable for commercial purposes due to different productivity problems, poor quality and commercial handling problems (Dorantes *et al*., 2004). Therefore, the identification and utilization of better varieties suitable for commercial purposes are necessary. A substantial quantity of avocado fruit is being wasted in many parts of

Kerala due to the lack of awareness on the health benefits of the fruit. But now, the demand for avocado is increasing which is evident from the data of total avocado import by India worth USD 140,000 in 2015-16 and worth USD 380,000 in 2016-17. In 2017-18, it increased by 70.33 per cent to USD 650,000 (Ians, 2018).

Depending on cultivar and geographic location, dry matter, picking date, fruit size, and oil content are the major characteristics that determine avocado fruit maturity indices. Lee *et al*. (1983) mentioned that the oil content in the mesocarp with minimum value of 8 % can be considered as the best avocado ripeness indicator even though its determination is complex. So dry matter is the most widely used index which can be determined in simple, fast and safe method and it further depends on the fatty acid content. Burdon *et al*. (2017) mentioned that the traditional maturity index for avocado fruit is dry matter which determine the eating quality so that the mouth feels sufficient oily rather than watery; however the appropriate physiological maturity is more associated with the preclimacteric period or the time taken to ripen. Pisani *et al*. (2017) mentioned that in practical, avocado fruits can be considered as mature when it stopped increasing size.

Avocado is one of the most nutritive among fruits in the New World, known as 'butter fruit' and 'green gold'. Avocado fruit pulp is rich in nutrients with an average amount of 17.34 g of fat, 2.08 g of protein, 2.72 g of fibre and 6.94 g of carbohydrate in 100 g of fresh pulp (Tucunduva, 2002). Avocado is a good source of essential nutrients such as protein, fat, vitamins, minerals such as calcium, potassium and iron. Also avocado oil contains monounsaturated fat which varies depending on the varieties (Orhevba and Jinadu, 2011). Being an important natural source of monounsaturated fatty acids with abundance of oleic acid and low content of saturated fatty acids, which lowers bad cholesterol (low density lipoprotein), total cholesterol and triglycerides in body (Salgado, 2005), avocado becomes an excellent source of beneficial fat (Ozdemir and Topuz, 2004). Oil extracts from avocado fruit pulp have similar fatty acid composition with that of olive oil, especially in oleic acid content (Mooz *et al*., 2012).

Avocado is a climacteric fruit which matures on the tree but ripens only after harvest. Avocado must be mature enough to ripe properly which depends on the dry matter content, oil content and texture. Avocado can remain on the tree for months which can be utilized by commercial growers for better marketing; but if remained too

long, get fallen off to the ground (FAO, 2002). The unique growth and development of avocado fruit, such as completion of maturation after harvest, followed by higher respiration and ethylene production leading to high perishability of the fruits under environmental conditions (Duarte *et al*., 2016). As avocado ripens within five to six days after harvest in ambient conditions and perish quickly, it results in huge post harvest loss by the rejection of fruits due to poor fruit quality standards.

Munhuweyi *et al.* (2020) reported that shelf life of avocado can be extended up to 6 weeks with the retention of firmness under controlled atmospheric storage of 5–12 ^{o}C; 95 % RH, 2–5 % O_2 and 3–10 % CO_2. Avocado fruit pulp is very sensitive to oxidative browning and even minimal thermal process would cause off flavour, bitterness and discolouration. Generally, freezing is also detrimental to the texture and flavour of the fruit, which can be turned to an acceptable product with application of quick freezing methods, along with pretreatment of acidulants such as ascorbic acid (Pauker *et al*., 1992). Addition of food additives such as sucrose improves overall quality of the product while compounds such as potassium sorbate and sodium benzoate have antimicrobial effect (Khan *et al*., 2014). Freeze drying attains 2.5 % lower moisture content than fresh sample and thereby, lowering water activity which curbs the enzymatic activity and microbial growth with the combined action of freezing and vacuuming (Owusu, 2011).

The present study is focussed on characterisation of the horticultural traits, biochemical and nutritional attributes of avocado fruit accessions cultivated in Kerala, looking forward to identifying promising genotypes which could help in spread and subsequent utilization in processing sector. In this background, present study titled "Post harvest characterisation and management of avocado (*Persea americana* Mill.) was undertaken with the following objectives:

1. Characterisation of avocado accessions
2. Effect of shrink packaging and storage temperature on quality and shelf life of avocado
3. Effect of food additives on quality of frozen avocado during storage
4. Effect of food additives on quality of avocado pulp and avocado fruit powder

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Characterisation of avocado accessions

2.1.1. Avocado accession

Avocado (*Persea americana* Mill.) is the only fruit crop belongs to the family Lauraceae, which is being cultivated in tropical and subtropical climates. As a polymorphic tree, there are about 50 genera and 3000 species in avocado that referred to the three botanical groups or horticultural races. All the three races of avocado have been adapted to the tropical and sub-tropical conditions in India. Even though the agro-climatic conditions in India are favourable, avocado is not cultivating in any commercial plantations. It is grown in higher altitude regions such as northeastern state like Sikkim and in southern tropical states like Tamil Nadu, Kerala, Karnataka, and Maharashtra. In Kerala, the cultivation is mainly focussed in Wayanad and Idukki districts with higher elevations (Tripathi and Shanker, 2014). In Kerala the cultivation started in the late nineties in Regional Agricultural Research Station, Ambalavayal, Wayanad. Major cultivars reported as being grown in Kerala included Fuerte, Purple Hybrid, Kallar Round and Pollock and presently due to the peculiar flowering habit which promotes outcrossing and the seed propagation methods followed in avocado result in wide variability among the genotypes. Inadequate characterisation and identification of these genotypes result in the lack of awareness, improper utilisation and insufficient post harvest management of avocado. The main objectives of the study were to characterise avocado accessions collected from different parts of Kerala and to evaluate the effect of post harvest management practices and its potential for value addition.

2.1.2. Characterisation

Three horticultural races of avocado such West Indian (*P. americana var. americana*), Guatemalan (*P. americana var. guatemalensis*) and Mexican (*Persea americana var. drymifolia*) and its hybrids have been adapted to tropical and sub-tropical conditions in India. International Plant Genetic Resources Institute (IPGRI) provided descriptors for avocado based on its varying morphological characteristics of trees, fruits and seeds as a whole, which indicates the availability of widely varying avocado genotypes.

Fruit shape may vary from round, pyriform or oblong, with varying fruit skin texture and colour. The skin of the fruit may be pliable to brittle, smooth to rough, and

green-yellow, reddish-purple, purple, or black in colour. The fruit flesh colour close to the skin and seed varied from greenish yellow to bright yellow, respectively with buttery texture on ripening in good varieties, but in poorer cultivars may be fibrous. Avocado fruit weight ranged from 150 g to more than 1.50 kg (Ayala-Silva, 2019).

Avocado has high nutritional density, rich in protein (up to 4%) and fat (up to 30%), but low in carbohydrate, with similar fatty acid composition as that of olive oil having higher concentration of monounsaturated fatty acids. Thus it can be used as a safe energy source for diabetic and cholesterolemia patients. Avocado has high energy value besides being a reservoir of vitamins and minerals attribute to its high antioxidant potential and become a boon for patients with cancer, CVD and other chronic degenerative diseases (Tripathi and Sanker, 2014).

The quality perception characteristics of avocado fruits can be determined based on its physical, biochemical and microbiological factors. Among the physical quality characteristics, size, weight and various visual specifications are considered as the important factors for fruit acceptance. The chemical factors include metabolic and nutritional profile of the fruits, from which the healthy consumption behaviour give importance to unsaturated fatty acids, antioxidants, etc. The fruit quality is determined by several pre-harvest and harvest factors and should be complemented with appropriate post harvest management practices, since at this stage fruit quality cannot improve but can be maintained (Ramírez-Gil *et al*., 2019).

2.1.2.1. Horticultural traits

Avocado is commonly called as butter pear because of its peculiar shape and the smooth texture of the pulp (Araújoa *et al*., 2018).

Liu *et al*. (1999) reported that early fruit growth was affected by fructose, glucose and D-mannoheptulose accumulation. In avocado, larger fruit size is due to the carbon accumulation and storage in which soluble sugars are the important carbon source contributing to the increase in fruit biomass. There are many morphological diff erences among the varieties and the most prominent trait is the colour of the fruit peel during ripening (Yahia and Woolf, 2011). Maturation in the tree is determined by the percentage of dry matter, which is reversely proportional to the percentage of moisture (Araujoa *et al*., 2018).

Avocado fruit weight varied from 120 g to 2.5 kg with smooth or rough surface; thin or thick skin; and pyriform, obovate, clavate or spheroid berry shape (Morton,

2004). Since avocado is growing in different tropical and subtropical regions and due to the cross pollination, fruits with different shapes, flavours, textures, colours and smells are formed, resulted in numerous varieties. Most popular and marketing fruit varieties, Hass and Fuerte belong to Guatemalan-Mexican hybrid race (Litz *et al*., 2007). Avocado fruits attained physiological maturity and remained on the tree for months until harvest and ripening happened only after harvest (Yahia and Woolf, 2011). Avocado is rich in high value oil of emerald green colour where higher oil content was reported in ripe fruit than the unripe fruit (Gatbonton *et al*., 2013).

Avocado fruit is pear-shaped, oval or round, with nearly 7.5–33 cm long and up to 15 cm wide. Fruit skin or peel is yellow-green, deep-green or a very dark-green, reddish or dark purple coloured sometimes speckled with tiny dots. The skin or peel may have up to 1/4 inch (6 mm) thickness with smooth or pebbled, glossy or dull, thin or leathery, pliable or brittle in nature. Immediately beneath the skin, flesh is thin, soft and bright-green in colour, and to inner portion flesh is entirely pale to rich-yellow or nutlike in colour with buttery or pastose or watery texture (Araujoa *et al*., 2018).

2.1.2.2. Biochemical characterisation

Avocado is considered as the most nutritive fruit of the New World to human diet, which has high nutritional content with health benefits of unsaturated fatty acids, insoluble and soluble fibres and proteins than many other fruits. Werman and Neeman, (1986) reported that maximum oil content as well as its oleic acid content were obtained in the peak ripening period. Avocado pulp contains moisture content of 67 to 78 %, lipid content ranging from 12 to 24 %, carbohydrate content from 0.8 to 4.8 %, protein content from 1.0 to 3.0 %, ash content ranging from 0.8 to 1.5 % and fibre content of 1.4 to 3.0 %. The most important nutritional factor is the lipid with monounsaturated fatty acids (predominantly oleic acids), which reduce the undesirable low-density lipoprotein (LDL) and increase the beneficial high-density lipoprotein (HDL) in blood (Cowan and Wolstenholme, 2016). Avocado is one of the finest salad fruits. Currently there are many patented products of avocado pulp or avocado oil in foods, cosmetics and medical field. But avocado is not considered as commercial fruit crop in India.

2.1.2.2.1. Total Soluble Solids

Total Soluble Solids (TSS) are one of the major biomass components in fruits during the early rapid development stage. Bower and Cutting (1988) mentioned that avocado fruit do not ripen on the tree and the reduction in sugar is a physiological prerequisite for fruit ripening. When the fruits are removed from the tree, the supply of

sugars from shoots reduced and initiated ripening. Liu *et al.* (1999) reported that TSS content lowered in ripe avocado fruit flesh during storage for 3 to 6 weeks at 1 and 5 ºCthan the unripe fruits in ambient condition. It was observed that the unripe fruit flesh without storage and ripe fruit stored at 20 ºC had 10 % and 8 % TSS concentrations, respectively. Decrease in the TSS in ripe fruits might be due to the reduction in carbohydrates such as glucose, fructose, sucrose and other sugar compounds. When avocado fruits attain minimum maturity stage with about 20.80 % dry weight, flesh accumulates oil instead of TSS or sugar.

2.1.2.2.2. Titratable acidity

With the maturation of fruits, organic acids are metabolized into non-acidic compounds due to respiration and thereby reduced the acidity. Vinha *et al*. (2013) observed acidity of 1.07±0.02 % in fruit pulp of Algarvian avocado variety 'Hass'. Defilippi *et al*. (2015) reported that the organic acid profile of Hass avocado such as tartaric acid, malic acid, citric acid and ascorbic acid, decreased with the advance of ripening, which was predominantly due to the decrease of malic acid and can be correlated to the decrease of titratable acidity during ripening.

2.1.2.2.3. Total carbohydrates

In avocado fruits, a high energy input was required for the development of high oil content and large seed which was obtained from carbohydrates derived from photosynthesis (Wolstenholme, 1986). During fruit storage, reserve carbohydrates acted as an energy source for the respiration and ripening (Kozlowski, 1992). Liu *et al*. (1999) reported that decrease of carbohydrates in the avocado fruit peel and flesh from harvest maturity to ripe stage and during post harvest storage in cool chambers at 1 or 5 ºC was due to the use of sugars for respiration as energy source. The decrease in carbohydrates such as glucose, fructose, sucrose and other sugar compounds resulted in the decrease in TSS of flesh during fruit ripening. Tesfay (2009) reported that avocado fruit edible portion contained C7 sugars predominantly D-mannoheptulose, an important antioxidant.

2.1.2.2.4. Total protein

USDA (2011) referred that protein content in avocado pulp was in the range of 1 to 3 %. Oliveira *et al*. (2013) reported protein content in a range of 0.74 % and 1.9 % in eleven varieties of avocados such as Ouro Verde, Wagner, Campinas, Paulistinha, Fortaleza, Pedroso, Margarida, Hass, Fortuna, Quintal, and Reis. Algarvian avocado

var. ‘Hass’ contained 1.82±0.07 % protein content as reported by Vinda *et al*. (2013). Tripathi and Shanker (2014) reported protein content of 1.35 % in the edible portion of avocado fruit variety TKD-1. Krumreich *et al*. (2018) reported about 1.7 ± 0.2 % protein content in avocado pulp of Breda variety.

2.1.2.2.5. Total fat

The avocado fat is similar to olive oil in composition which contributes to its importance in therapeutic purposes and for the preparation of cosmetics. Liu *et al*. (1999) reported that as fruit matured and slowed the growth, the amount of accumulated sugars decreased, which can be correlated with increase in oil deposition. Jorge *et al*. (2015) observed about 7.7 and 8.8 % oil yield in the fruit pulp of Margarida and Hass avocado variety. Krumreich *et al*. (2018) reported 16 % fraction of lipids out of 72 % pulp of avocado fruit.

2.1.2.2.6. Vitamin C

Decrease in vitamin C is associated to the postharvest conditions such as long-term storage, higher temperature, low relative humidity, physical damages and chilling injury of fruit (Lee and Kader, 2000). Ascorbic acid acts as a strong antioxidant in the lipid phase of avocado pulp (Soliva *et al*., 2002). USDA (2011) mentioned that 30 g and a half of avocado fruit contained about 2.6 mg and 6.0 mg of vitamin C, respectively.

2.1.2.2.7. Calcium

Cutting *et al*. (1992) reported calcium content in Reed avocado variety in the range of 8.9 to 13 mg/100g. Tripathi and Shanker (2014) reported calcium content of 10 mg/100g in the edible portion of avocado fruit. Archana (2019) reported calcium content in avocado cultivars collected from Ambalavayal such as Fuerte, Purple Hybrid, Kallar Round and Pollock as 9.15, 9.15, 8.65 and 9.30 mg/ 100g, respectively.

2.1.2.2.8. Potassium

Potassium content in avocado is considered as highest in tropical and non-tropical fruits and vegetables and is twice than the potassium content in banana. USDA (2011) mentioned that 152 mg and 345 mg of potassium was presented in 30 g and half portion of fruit, respectively and in Hass variety, it was in the range of 405 to 507 mg/100g. Archana (2019) reported potassium content in avocado cultivars collected from Ambalavayal such as Fuerte, Purple Hybrid, Kallar Round and Pollock as 343.33, 393.00, 384.76 and 394.47 mg/ 100g, respectively.

2.1.2.2.9. Iron

USDA (2011) mentioned iron content in Hass avocado variety in the range of 0.39 to 0.61 mg/ 100g of fruit pulp. Tripathi and Shanker (2014) reported iron content of 0.6 mg/100g of edible portion of avocado fruit. Archana (2019) reported iron content in avocado cultivars collected from Ambalavayal such as Fuerte, Purple Hybrid, Kallar Round and Pollock as 0.403, 0.293, 0.283 and 0.320 mg/ 100g, respectively.

2.1.2.2.10. Total ash

Total ash content in the fruit is associated with the minerals composition of the fruit. Oliveira *et al*. (2013) reported it in the range of 0.83–2.40 % among eleven different varieties of avocado while Krumreich *et al*. (2018) reported low ash content of 0.6 % in the fruit pulp of Breda variety of avocado.

2.1.2.2.11. Total phenols

Phenolic compounds are bioactive compounds which act as the natural source of antioxidants that reduce free radical formation and scavenge free radicals (Heim *et al*., 2002).

Lidster *et al*. (1986) reported the presence of high amounts of phenolic antioxidants in the mesocarp of avocado which was responsible for the browning of the fruit pulp. Tesfay (2009) quoted that the higher concentration of free phenols acts as antioxidants and improves the post harvest fruit quality and health benefit of the fruit. Krumreich *et al*. (2018) reported phenolic compounds in Breda variety of avocado as 97.27 mg gallic acid kg^{-1}. Archana (2019) reported total phenols content in avocado cultivars collected from Ambalavayal such as Fuerte, Purple Hybrid, Kallar Round and Pollock as 62.30, 63.23, 68.60 and 63.14 mg/100g respectively.

2.1.2.2.12. Total flavonoids

Vinha *et al*. (2013) reported flavonoid content of 21.90 mg/100g in Algarvian avocado (Hass variety in Algarve region of Portugal). Archana (2019) reported total flavonoid content in avocado cultivars collected from Ambalavayal such as Fuerte, Purple Hybrid, Kallar Round and Pollock as 18.37, 21.07, 18.13 and 20.73 mg/100g respectively.

2.1.2.2.13. Fibre

USDA (2011) referred about 1.4 to 3.0 % fiber in avocado pulp. Oliveira *et al*. (2013) reported it in the range of 2.95–8.15 % among eleven different varieties of avocado. Tripathi and Shanker (2014) reported crude fibre content of 1.8 g/100g of

edible portion of avocado fruit. Krumreich *et al*. (2018) reported low fiber content of 1.6 ± 0.4 % in the fruit pulp of Breda variety of avocado while Daiuto *et al*. (2010) reported 1.62 % of fiber in Hass variety.

2.1.2.2.14. Oleic acid

Oleic acid was found to be the most dominant fatty acid in avocado with 43.37 % and 41.91 % yield in soxhlet extraction of freeze dried avocado pulp using hexane and ethanol, respectively (Gatbonton *et al*., 2013). Oleic acid helps to lower serum cholesterol levels and low-density lipoproteins in the human body (Gatbonton *et al*., 2013). Owusu (2012) mentioned that oleic acid prevented stroke, breast cancer and lowers cholesterol levels in blood. In commercial avocado oil, highest concentration of oleic acid reported was about 73.88 g/100g (Jorge *et al*., 2015) and in Hass variety it was reported as 53.00 % (Donetti and Terry, 2014).

2.1.2.2.15. Antioxidant activity (DPPH, ABTS, FRAP)

Antioxidants can be defined as compounds that protect cells against the damaging effects of oxygenated and nitrogenated free radicals, formed in oxidative processes and it acts by inhibiting or decreasing the effects of free radicals which are responsible for triggering various types of chronic-degenerative diseases. Antioxidants can be obtained through food especially which is rich in vitamin E and C, carotenoids, phenolic compounds, etc.

Avocado can be considered as a functional food with nutraceutical antioxidants, such as vitamin E or tocopherols, lutein and glutathione composition three times more than any other fruits. Antioxidants neutralize free radicals which damages heart and cells causing aging and cancer. Tesfay (2009) mentioned that antioxidant content was low in the mesocarp than that of exocarp and seeds during the growing period. In food containing fats and oils, autoxidation of unsaturated fats results in rancidity which can be prevented partially or completely by the addition of antioxidants such as ascorbic acid (Owusu, 2012). Fat-soluble vitamin E is one of the most important antioxidant which protects the membrane integrity (Munteanu, 2021).

DPPH assay determines free radical scavenging activity of samples related to the hydrophilic polyphenols based on the antioxidant reaction with an organic radical. It is based on the neutralisation of DPPH radical by donating electrons from the antioxidants which is indicated by the discoloration of DPPH from deep purple to pale yellow at 517 nm. (Brand-Williams *et al*., 1995)

ABTS assay depends on the hydrogen atom donating ability of both hydrophilic and hydrophobic (fat soluble) compounds in which antioxidants in the sample reduce $ABTS^{+}$ to stable compound in which potassium persulphate acts as the oxidant. Degree of discoloration of blue-green chromophore of $ABTS^{+}$ is measured by the sudden decrease in the absorbance to 734 nm indicates the antioxidant activity (Benzie and Strain, 1996).

FRAP assay is based on the ability of samples to donate electrons in order to reduce Fe^{+3}-TPTZ complex to a blue coloured Fe^{+2}-TPTZ complex. In FRAP assay, antioxidants with chelating property reduce Fe^{3+} to Fe^{2+} or ferricyanide to ferrocyanide to yield Prussian blue colour (Vargas-Ortiz et al., 2016).

Wang *et al*. (2012) reported antioxidant activity of 60.29 % in Hass variety. Vinha *et al*. (2013) reported DPPH antioxidant activity of 23 % in the fruit pulp of Algarvian avocado, variety Hass. Krumreich *et al*. (2018) reported an antioxidant activity of 78.90 % in Breda variety. Zouheira *et al*. (2018) reported IC_{50} values of ten Hawaiian and Cameroonian avocado varieties by DPPH, ABTS and FRAP methods, which was in the range of 122.45 to 469.05 µM, 66.06 to 427.25µM and 56.04 to 253.23 mM catechin equivalent, respectively

2.2 Effect of shrink packaging and storage temperature on quality and shelf life of avocado.

As a climacteric fruit with high rate of respiration and ethylene production, microbial attack and deterioration of colour and texture by endogenous enzymes are the major problems encountered in avocado post harvest management. Avocado is chilling sensitive, result in both flesh and skin damages at low temperature below 5 ºCand limit storage life. Modified atmosphere packaging and low temperature storage extend the shelf life of avocado by reducing the respiratory and metabolic activities and thereby reduce the ripening of fruits. (Spalding, 1976)

2.2.1. Surface sanitisation by ozonisation

Ozone treatment in the fruit preservation is gaining importance as part of reducing the harmful effects of chemicals and its residues. Ozone is approved as GRAS (Generally Recognized as Safe) substance and it preserves the fruit quality by killing decay causing and pathogenic microorganisms as well as control ripening by degrading the ethylene gas. Preservative effect of ozonisation is based on the oxidizing power of

highly reactive oxygen released from the unstable ozone molecules in water. Karaca and Velioglu (2007) elucidated the potential of ozone in destroying microorganisms, mycotoxin and pesticide degradation, maintaining the quality of fruits and inhibitory action on polyphenoloxidase, free of any residual effect. Trimble (2020) reported the significant effect of ozone treatment in reducing the ethylene production and respiration rate and reducing the avocado fruit loss to 2 to 3 % while the loss in control was 7 %.

2.2.2. Calcium chloride ($CaCl_2$)

Fruits treated with calcium chloride were observed as microbiologically most stable along with reduced respiration rate and preserved mechanical properties (Yadav and Singh, 2014). Calcium chloride act as an osmotic agent which helps to increase firmness, preserve texture and prevent browning of fruit pieces during storage. Calcium chloride salts have texture reinforcement and cell turgor retention ability and minimised tissue damage during processing (Giannakourou *et al*., 2020).

Minh *et al*. (2019) reported that post harvest infiltration of calcium chloride into avocado delayed ripening for 2-3 days and extended the shelf life with retaining constituents like ascorbic acid and maintaining sensory qualities like sweetness, colour, odour and hardness of fruit.

Arlai *et al*. (2014) observed that okra blanched with calcium chloride solution at 0.5 % w/v for 90 seconds improved moisture and fat content and sensory qualities including hardness, crispness and colour.

2.2.3. Shrink packaging

Rao and Shivashankara (2015) reported that individual shrink packaging of mango cvs. ‘Alphonso’ and ‘Banganapalli’, stored at 8 ºCand ambient conditions extended the storage life, retained nutritional quality including antioxidants and maintained good surface skin colour, firmness and all organoleptic qualities in acceptable range. The fruits kept normal respiratory pattern with climacteric peak in the CO_2 and ethylene production even after removed from low temperature and unpackaging. Kiharason and Isutsa (2019) mentioned that packaging of fruit had an important role in preservation by reducing the rate of moisture loss and inhibiting microbial growth by forming anaerobic environment.

2.2.4. Polyolefin film

Polyolefin films are high shrink force resisting films with extremely durable and versatile properties. Polyolefin films have high tensile strength and clarity facilitating high speed packaging with anti-fog formulations and reduce moisture vapour

transmission rate and can be customized according to the product. Shrink films are capable of enhancing the shelf life of perishable products and protecting the sensorial qualities like smell, colour, appearance *etc*. FDA approved it as food safe material and is durable and recyclable (St. Worcester, 2021)

Alehegn *et al*. (2017) reported that avocado fruits packaged in plastic material had highest excellent preference on aroma, firmness, flavour, skin colour and better marketability with longer shelf life, higher pulp to peel ratio and lesser decay percentage and physiological loss in weight when compared to the packaging methods like carton (Corrugated Fibre Board), enset leaves and open ground (control).

2.2.5. Storage temperature

Avocado fruit stored in air for more than 30 days reduced the ripe fruit quality (Dixon, 2003) and as a chilling sensitive fruit, both flesh and skin disorders may occur at low temperature (Hofman *et al*., 2002). The combination of controlled atmosphere with refrigerated storage maintained good ripe fruit quality for 6-8 weeks (Burdon *et al*., 2008). Low temperature storage extended the shelf life of avocado by reducing the respiration rates, ethylene evolution, softening and colour change (Perez *et al*., 2004).

As a climacteric fruit which ripens after harvest, storage temperature and storage time influence the post-harvest behaviour of avocado fruit. According to Honorio and Moretti (2002), the temperature for storage of avocado fruit varied from 5 to 12 ^{o}C, while Chitarra (2005) reported that, it can be varied from 4.5 to 13 ^{o}C. According to Munhuweyi *et al*. (2020), low temperature storage (5–13 ^{o}C) had prime importance to extend the postharvest quality of avocado.

Refrigeration can be considered as a quality preservation method which retards the speed of cell metabolism and fruit senescence. Avocado fruit cv. Hass stored for 4 weeks at 5 ºC had sound fruit with overall better quality while fruit stored at 2 ºC had severe stem-end rot and brown patches and flesh discolouration (chilling injury) which were increased with storage durations (Dixon *et al*., 2004).

According to Bhande *et al*. (2008), increase in post harvest life of fruits at low temperature storage attributed to the slowdown of respiration and decrease of enzymatic activities due to lower utilization of storage compounds. Meyer and Terry (2010) mentioned that exposing avocado fruit to a temperature lower than the critical threshold temperature of 10-15 ºC may cause irreversible damage to the cells resulted in pulp spot and chilling injury.

2.2.6. Shelf life

Shelf life is the useful storage period of a food after which it develops characteristic undesirable changes in appearance, texture, taste and aroma due to biochemical reactions, microbial reactions, enzymatic browning and physical changes like loss of texture (Owusu, 2011). At optimum shelf life, the product can be considered as safe to consume, retains nutritional and sensorial qualities after which it is unfit for consumption without adequate sensorial attributes.

2.2.7. Physiological Loss in Weight

Chitarra (2005) mentioned that due to the combined effect of breathing and perspiration during storage, weight loss is inevitable for perishable products like avocados even under ideal storage conditions. Vieites *et al.* (2012) reported that loss of weight did not exceed 2 % of the initial fruit weight in cold storage while under ambient condition loss of weight was more amplified.

2.2.8. Respiration rate

The respiration rate of avocado fruit exhibited a typical climacteric pattern during storage. Since the primary function of respiration is the production of energy and metabolite intermediates, is a degradative process which lowers the storability of fruits. Vieites *et al.* (2012) observed a higher evolution of CO_2 in avocado fruits stored at room temperature than those kept under refrigeration and the respiratory peak was noticed at 9^{th} day of storage. Pathirana *et al*. (2013) mentioned that the low O_2 levels in the package inhibited the peak of respiration rate in which the respiratory peak was reported after 4 days of ambient storage and decreased the respiration rate afterwards. Alamar *et al*. (2017) mentioned that post harvest storage of avocado fruits exposed to controlled atmosphere storage (CAS) at 20 ºC reduced respiration rate and retained fruit firmness with some internal discolouration noticed after 7 days of withdrawal from CAS, while at 5 ºC fruit skin and flesh colour were retained more than control.

2.2.9. Ethylene evolution rate

Ethylene, a phytohormone, has pivotal roles in ripening and senescence of climacteric fruits. The ethylene evolution rate of avocado fruit exhibited a typical climacteric pattern during storage. Low O_2 atmosphere influences post-harvest physiology and quality of fruit either directly or indirectly through altered CO_2 and ethylene production rates (Kader, 1989). Pathirana *et al*. (2013) mentioned that the low O_2 levels in the package suppressed ethylene production and delayed the peak of

ethylene by the inactivation of ACC synthase and reduced conversion of ACC to ethylene. Mendieta *et al*. (2016) reported that CAS resulted in more light, vivid, firm fruits with reduced rate of senescence and an extended shelf life of 2 days regarding the retention of firmness which is mainly due to the lower production of endogenous ethylene.

Chilling injury symptoms in avocado can be reduced by storing the fruits in modified atmosphere storage (MAS) with high CO_2 and reduced ethylene concentration (Meir *et al*., 1997). Pesis *et al*. (2002) studied the effects of ethylene in avocado on the development of internal chilling injury (CI) symptoms during prolonged cold storage and observed that higher ethylene levels caused mesocarp discoloration, stem-end rot and increase in PPO activity. MAS reduce ethylene concentration around the fruit by the direct absorption of ethylene or elimination of ethylene forming reaction which leads to lower CO_2 and higher O_2 concentrations, thereby slow down the ripening process, reduce mesocarp discoloration and decay (Pesis *et al*., 2002).

2.2.10. Texture

Fruit firmness is the universally accepted measure of fruit ripeness or maturity which helps to determine the best picking time, and to monitor fruit ripening and softening of fruit during storage (Flitsanov *et al*., 2000). During ripening, fruits may soften and become too soft at the overripe stage. The changes in firmness can be detected by pressing on the fruit surface or by using devices like pressure analysers. Maximum force required to rupture the sample is measured by pushing a cylindrical metal probe into the sample to a given depth.

Texture is an important quality attribute and sensory characteristic of the fruits which provides resistance to transportation shock, attack of microorganisms however, loss of firmness is inevitable during post-harvest. In Fuerte avocado, Vieites *et al*. (2012) observed that at room temperature the firm texture was decreased gradually while under refrigeration firmness was increased and drops only after the respiratory peak. Pisani *et al*. (2017) mentioned that the ripe avocado fruits attained an optimum firmness of 20-30 N which can be considered as one of the postharvest ripe fruit quality.

2.2.11. Decay

Skin pitting, scalding, water soaked areas, uneven ripening, blackening, off flavour development and decay were the external symptoms of chilling injury in avocado while internally it appeared as grey pulp, pulp spot and vascular browning

(Yahia and Woolf, 2011). Udara *et al*. (2019) mentioned that in avocado decay symptoms were predominantly observed as black spots in epidermis and black or brown discolorations of mesocarp. Pulp spot and chilling injury are common disorders in avocado stored under low temperature, which forms dark spots in the flesh and blackening around the vascular bundles after cutting or bruising implicating the enzyme polyphenol oxidase (PPO) (Munhuweyi *et al*., 2020).

2.2.12. Post harvest disease incidence

Avocado fruit quality during storage was mainly compromised by postharvest fungal diseases such as anthracnose and stem-end rot caused by *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae,* respectively (Bowen *et al*., 2018). During storage, fungi cause rots and blemishes to edible parts and reduced appearance and market value. Udara *et al*. (2019) mentioned that avocado was more susceptible to post harvest losses due to its smooth skin, higher rate of metabolism, respiratory rate and ethylene evolution rate after harvesting. Thus it became more susceptible to mechanical damages, chilling injury (CI), physiological disorders, decay and insects infestation.

2.2.13. Biochemical evaluation

2.2.13.1. Total Soluble Solids

In avocado at minimum maturity stage, fruit flesh began to accumulate fats/ lipids and hindered TSS accumulation and decreased carbohydrate content in fruits (Liu *et al*., 1999). While Kassim and Workneh (2020) reported that the increase in hydrolysis of carbohydrates stored within the avocado into soluble sugars may increase TSS and reduce shelf life of the fruit.

2.2.13.2. Titratable acidity

In Fuerte avocado, Vieites *et al*. (2012) observed that titratable acidity increased up to the respiratory peak and decreased thereafter and also noticed that the rise was faster under room temperature (24 ± 1 ^{o}C) than refrigerated condition at 10 ± 1 ^{o}C. Respiratory behaviour of avocado resulted in the reduction of titratable acidity in the fruits kept at room temperature (24 ± 1 ^{0}C) and refrigerated temperature (10 ± 1 ^{o}C) and 90 ± 5 % relative humidity for 15 days.

2.2.13.3. Total carbohydrates

Major total carbohydrates present in avocado consist of heptose sugar (D-mannoheptulose, and perseitol) and hexose sugars (glucose and fructose) which were used as carbon energy source for respiration and decreased subsequently during post harvest storage (Liu *et al*., 1999)

2.2.13.4. Total protein

Blakey *et al*. (2012) reported increase in soluble protein of Hass avocado from 17.8 to 34.8 mg^{-1} g DW from day 2 to 16 with the increase in enzyme synthesis with the increase of ripening process.

2.2.13.5. Vitamin C

Anbessie *et al.* (2013) reported degradation of ascorbic acid with increase in storage time and temperature with a per cent retention of 70.3, 55.7 and 31.7 at 5, 10 and 15 ^{o}Cafter ten weeks of storage.

2.2.13.6. Total phenols

Vieites *et al*. (2012) observed an increase in total phenolic compounds up to the respiratory peak and decrease with the senescence thereafter, for avocado fruits kept in both ambient and refrigerated storage conditions. The lowest total phenolic content were seen in fruits stored under refrigerated conditions. Pathirana *et al*. (2013) reported an increase in the total phenolic content of untreated avocados from 0.45 to 0.59 mg GAE/g DW over the storage period of 28 days while under cold storage total phenolic content significantly reduced in the avocado mesocarp. Celli *et al*. (2016) mentioned that general freezing technique has little deleterious eff ect on the phenolic content of fruits.

2.2.13.7. Total fat

Pathirana *et al*. (2013) reported that in avocado fruits (cv. Fuerte) stored for 2 weeks at 20 ^{o}C, a significant decrease in unsaturated fatty acid and significant increase in saturated fatty acid and 2 % decreased in oleic acid were observed which could be related to the oxidative degradation of fatty acids.

2.3 Effect of food additives on quality of frozen avocado during storage

2.3.1. Quick frozen avocado slices

Freezing process increases solute concentration and thereby damages may occur in cellular structures. Freezing crystallizes water in fruits, which constitutes about 85–

90 % and reduces water activity (a_w), thereby retards biochemical changes, microbial growth and prevents nutritional loss during storage. In quick-freezing, the freezing process progress quickly from the surface to the centre until the centre of the fruit attains -18°C. Nikapitiya and Illeperuma, (2006) reported that avocado fruit slices spoiled after 3 days in storage without any treatments while those in package of 0.05 mm and 0.075 mm thickness with moisture absorber and stored at 8°C had shelf life up to 10 days. It is due to the potential of MAP under low temperature to reduce the rate of respiration and browning of the avocado fruit slices. Chassagne-Berces *et al*. (2010) reported that in apples, air-blast freezing caused least texture degradation while Shah *et al*. (2020) observed the maintenance of microstructure and physicochemical quality of litchi fruits under immersion freezing along with air-blast freezing, stored at −18 ºC for 6 months.

In frozen fruit products, texture can be affected by volume expansion during freezing and recrystallization, causing damage to cell membranes during ice crystals formation and empty space development whereas fast freezing maintained the texture of frozen products. Souza *et al*. (2014) reported that samples after freezing had less hardness and compact structure by reducing the adherence with increased porosity and reduced density. During freezing, cells and tissues may be protected from damage. Rapid freezing reduces the loss of water from the cells and also reduces the cell wall damage due to smaller ice crystals (Dawson *et al*., 2020).

2.3.2. Sucrose

Sucrose is a non-reducing sugar with very low disaccharide bond energy and good potential in lowering water activity and higher moisture diffusivity (Giannakourou *et al*., 2020).

Delgado and Rubiolo (2005) reported that sucrose pretreatment retained the tissue integrity of frozen strawberries during storage. Dermesonlouoglou *et al*. (2007) reported that in watermelon, osmotic dehydration reduced water activity and retained sensory characteristics similar to fresh product while the freezing and low temperature storage had significant beneficial effects on lycopene content, colour, texture, and sensory properties compared with untreated samples.

Osmotic Dehydration (OD) is the preservation method which partially removes water from fruits and vegetables immersed in hypertonic solution of salt or sugar which improved the stability and quality of the perishable products (Khan *et al*., 2014). Ferrando and Spiess (2001) mentioned that in osmotic treatment plant material exposed to concentrated aqueous solutions of salt or sugar which resulted in osmotic pressure

and simultaneous release of water from the product and diffusion of solute to the product. Zzaman *et al.* (2020) mentioned that immersion pre-treatment increased solid gain and minimised the quality change in dried pineapple slices.

2.3.3. Ascorbic acid

As an antioxidant with reducing properties, ascorbic acid (vitamin C) within osmotic solutions was capable to avoid undesirable enzymatic browning (Giannakourou *et al*., 2020).

Cortés-Rodríguez *et al*. (2019) mentioned the most commonly used additives as antioxidants in the preparation of guacamole include ascorbic acid and citric acid. Moon *et al*. (2020) mentioned ascorbic acid and citric acid as acidulants which are widely used as anti-browning agents in food.

2.3.4. Preservatives (KMS, sodium benzoate, potassium sorbate)

Preservatives are additives which enhances the shelf life of foods by acting against harmful microorganisms and preventing fermentation and spoilage without causing any adverse effects to the consumer. Different chemical preservatives that are recommended to use in food include antimicrobials such as sodium benzoate, potassium metabisulphite, potassium sorbate and antioxidants like ascorbic acid. Rashmi *et al*. (2005) observed that in 60 ^{o}Brix syrup with 0.2 % critic acid and 700 ppm KMS (potassium metabisulphite) was the best combination of preservatives for the storage of osmotic dehydrated pineapple slices. Krebs *et al*. (2014) mentioned that potassium sorbate retarded yeasts and molds growth in yogurt, cheese, wine, dried meats and dehydrated products while sodium benzoate had bacteriostatic and fungistatic effect mostly in acidic foods. Meera (2019) reported that the highest sensory mean rank in pre-treated avocado was observed in those treated with solution comprising KMS (0.25 g), citric acid (0.1 g) and sugar (100 g). Rani and Tripathy (2019) reported that immersion pre-treatment of dried pineapple slices in potassium metabisulphite at 0.25 % concentration maintained better quality characteristics. Kassim and Workneh, (2020) reported that LDPE package maintained high moisture content, delayed ripening by minimizing the changes in TSS and viscosity of wax coated avocado fruits.

2.3.5. Subzero storage

Lopez-Malo *et al*. (1998) filled the guacamole in vacuum sealed containers and stored under frozen condition at -22 ^{o}C. Dawson *et al*. (2020) reported that freezing to lower temperature of -18 ºCor below ensured long term preservation of peaches with higher sensory quality and nutritional retention.

2.3.6. Biochemical evaluation

2.3.6.1. Total Soluble Solids

Kassim and Workneh (2020) mentioned that storage period and conditions influenced the quality factors like TSS. It was observed that TSS increased over a storage period of 28 days which was faster at ambient conditions and slower in wax coated LDPE packed avocados stored in low temperature. Increase in TSS may be due to the hydrolysis of carbohydrates into soluble sugars. Shah *et al*. (2020) reported that lychee slices treated with 0.1 % citric acid and 0.05 % KMS along with 50 % glucose was observed with highest TSS content, which was decreased during the storage of three months in transparent glass jars.

2.3.6.2. Titratable acidity

Kassim and Workneh (2020) reported that cold storage treatments at 4.5 to 5.5 ºC and 95 % RH maintained the quality of avocado such as acidity and improved shelf life and increase in the acidity was correlated with the avocado deterioration. It was observed that the increase in titratable acidity was faster under ambient condition (1.9 to 11.5 %) than low temperature storage (1.7 to 7.5 %) and in LDPE packaged fruits had low titratable acidity than unpackaged fruits. Shah *et al*. (2020) reported that lychee slices treated with 0.1 % citric acid and 0.05 % KMS along with 50 % glucose were observed with highest acidity, which was increased during 3 months of storage in transparent glass jars.

2.3.6.3. Total protein

Fuster *et al*. (1994) reported increase in soluble proteins of frozen kiwi slices during frozen storage which was explained as the result of mechanical damage by the ice crystals in tissue.

2.3.6.4. Vitamin C

Vitamin C (ascorbic acid) is a temperature-sensitive as well as water-soluble vitamin. Forni *et al*. (1997) studied the effect of osmotic dehydration followed by air dehydration on the quality of apricot cubes, frozen at −40 ºC (air speed of 4 m s^{-1}), and stored at −20 ºCfor 8 months. It was observed that discoloration and degradation of ascorbic acid depended on the pretreatment with sugar syrup, which lowered structural collapse and material density of the samples during drying. Mullen *et al*. (2002) observed that the vitamin C content decreased in fruits subjected to temperature fluctuations before freezing rather than fresh and immediately frozen raspberries at −30

^{o}C. Shah *et al*. (2020) reported that lychee slices treated with 0.1 % citric acid and 0.05 % KMS along with 50 % sucrose decreased the vitamin C content during 3 months of storage in transparent glass jars.

2.3.6.5. Total phenols

Ramirez-Martinez and Luh (1973) reported that frozen avocado packed in cans at 500 mm of Hg vacuum prevented the darkening for 3 months of storage at -26 ºCand became dark on exposure to air along with disappearance of extractable phenolic substances. De Ancos *et al*. (2000) reported that freezing had slight effect on the total phenolic content in raspberry. Dawson *et al*. (2020) reported that phenolic compounds subjected to oxidation during processing and storage, and loss of phenolic compounds was attributed to polyphenol oxidase (PPO) enzyme reactions.

2.3.6.6. Total carbohydrates

Wiley and Stembridge (1961) reported that starch content had significant effect in the texture of processed apple slices. Giannakourou *et al*. (2020) mentioned that osmotic treatment with low molecular weight carbohydrates did not significantly alter the sensory qualities of fruit tissue. Dermesonlouoglou *et al*. (2018) mentioned that osmotic treatment with disaccharides along with freezing had protective effect on proteins in the kiwifruit slices.

2.3.6.7. Total fat

Fat content in the fruit is based on the lipid metabolism, which may increase with the increase of temperature and heating time, resulted in high peroxide values in oils (Sarwar *et al*., 2016). Pauker *et al.* (1992) mentioned that oxidative deterioration is marginal in frozen avocado products and the stability of lipids in avocado could be due to the high contentof antioxidants such as tocopherols. It can also be enhanced by the addition of antioxidants like ascorbic acid, BHA or BHT.

2.3.6.8. Peroxide value

Pathirana *et al*. (2013) mentioned that peroxide value represented the formation of intermediate hydroperoxides, expressed in milliequivalents of active oxygen per kilogram of sample. It could be lowered by exposing the fruits in low O_2 levels under low-temperature storage conditions.

2.3.6.9. Polyphenol oxidase activity

Palou *et al*. (2000) reported inactivation of PPO in avocado guacamole by lowering the pH to 4.3 by using citric acid. Woolf *et al*. (2013) reported that avocado

fruit slices treated with high pressure increased PPO activity up to 30 %, might be due to the high release of enzyme during cell membrane breakdown and mentioned that PPO enzyme was more stable at neutral pH (7) or slightly higher (8).

2.3.6.10. Water activity

Maltini *et al*. (2003) classified that osmotic dehydration formed high moisture foods (0.99 to 0.95), reduced moisture foods (0.95 to 0.85) or intermediate moisture foods (0.85 to 0.65) based on water activity. Kiwi fruits with initial water activity of 0.990 ± 0.001 decreased in a higher osmotic (65 °Brix) solution which might be due to the increase in sugar concentration led to a lower free water availability and resultant reduction of water activity, in which low osmotic concentration allowed only slower rate of mass transfer (Brochier *et al*., 2019). Reduction in water activity minimized the microbial growth. The higher gain in soluble solids were observed in solution with higher osmotic concentration due to the counter diffusion of solutes in the osmotic solution to the food along with water diffusion. Ferrando and Spiess (2001) reported reduction in water activity from 0.952 to 0.891 of strawberry in sucrose solution which resulted in decrease of cell viability and also sugar concentration in the osmotic solution had significant effect on the membrane integrity.

2.3.6.11. Microbial load

Dekevich (2018) reported eight avocado related outbreaks and six recalls between 1998 to 2017 and 2010 to 2019, respectively which indicated its conducive properties for microbial growth such as high lipid and moisture content as well as low acid and carbohydrates content. The most commonly reported pathogens were *Salmonella*, *Shigella* and *Listeria.* Kassim and Workneh (2020) reported that LDPE package provided conducive environment for the microbial growth due to moisture condensation and loss of moisture from avocado fruit resulted in higher PLW.

2.3.6.12. Organoleptic evaluation

Kassim and Workneh (2020) reported that LDPE packaged avocado fruits under low temperature of 4.5 to 5.5 ºChad organoleptically appealing glossiness with higher percentage of marketability for 28 days of storage. Shah *et al*. (2020) reported that lychee slices treated with 0.1 % citric acid and 0.05 % KMS along with 50 % sucrose recorded highest sensory evaluation scores in colour, flavour, texture and overall acceptability, which was decreased during the storage of three months in transparent glass jars.

2.4. Effect of food additives on quality of avocado pulp and avocado fruit powder

2.4.1. Process standardization for preparation and storage of avocado pulp

2.4.1.1. Citric acid and ascorbic acid

Ospina *et al*. (2019) reported that citric acid, as an antibrowning agent, was capable to protect the avocado puree by reducing the PPO and POD activity and reducing colour change over time under vacuum packing and frozen storage conditions. Minh *et al*. (2019) mentioned the effect of citric acid and ascorbic acid as an antioxidant on the stability and sensory quality of avocado powder. In the study, it was observed that as the citric acid concentration increased, antioxidant activity in terms of tocopherol content was also increased significantly. Mujaffar and Dipnarine (2020) suggested the use of ascorbic acid and citric acid to prevent avocado pulp discoloration.

2.4.1.2. Preservatives

Cortes-Rodríguez *et al*. (2019) mentioned that among the most commonly used additives, sodium benzoate and potassium sorbate added as important preservatives in the preparation of guacamole.

Wali *et al*., (2012) observed that mango seabuckthorn blended pulp preserved with combination of potassium sorbate and potassium metabisulphite as well as sodium benzoate and potassium metabisulphite at 0.05 % concentration had obtained higher score for physico-chemical and sensory analysis.

2.4.1.3. Vacuum packed LDPE

Olaeta and Undurraga (1995) reported that better quality avocado pulp and slices was stored for 35 days by 40 % vacuum treatment in LDPE bags and it was concluded that a good preservation method of avocado pulp and slices was under cold storage in low density polyethylene bags using vacuum or modified atmosphere.

Castaneda-Saucedo *et al*. (2014) reported that vacuum packed freeze dried avocado pulp became porous and lightweight without any compaction or oil exudation during storage indicating the absence of cell damage during freezing. Since there is no direct exposure to ambient oxygen, the loss of biological value of mono and polyunsaturated fatty acids by oxidation becomes less and enhances the oil retention.

2.4.1.4. Glass jars

Durrani *et al*. (2011) preserved osmotic dehydrated carrot candy in glass jars and LDPE pouches for 6 months. Durrani and Verma (2011) concluded that glass jars were better packaging material for murabba in maintaining its physicochemical

properties, microbiological stability and sensory qualities during the storage period of 6 months. Hamid *et al*. (2017) reported that the quality of products retained significantly in glass jars under refrigerated conditions with minimum reduction in ascorbic acid, colour units, and other sensory qualities and increase in TSS, viscosity, pH, which was mainly due to the low thermal conductance, slower absorbance of heat and slower rate of chemical reactions.

2.4.1.5. Ambient and refrigerated storage

Owusu (2012) reported a shelf stability of avocado fruit spread for 47.50 days under refrigeration, otherwise which had short shelf life. Mujaffar and Dipnarine (2020) reported that products stored at 4 ºCwere microbiologically stable throughout the storage, maintaining all quality and sensory characteristics, but observed off-taste development in frozen avocado fruit pulp during defrosting due to tissue collapse.

Araujo *et al*. (2017) mentioned that the passion fruit pulp had lower soluble solids and acidity under both refrigerated and cold storage with longer storage period.

2.4.1.6. Pulp yield (%)

Tango *et al*. (2004) reported that the avocado pulp yield was observed in the range 52.9 to 81.3 % of the fruit weight. Nair (2018) found the pulp yield ratio of 86.20 % in Pollock, 75.40 % in Kallar Round, 75.11 % in Purple Hybrid and 71.30 % in Fuerte. Udara *et al*. (2019) mentioned that avocado fruit pulp content recovery was in the range of 52.90 to 81.30 % in which 25 % dry matter consists of lipids in major and carbohydrate in minor proportions.

2.4.1.7. Total Soluble Solids

TSS is a quality parameter associated to the texture and composition of fruit and in avocado, it predominantly includes soluble sugars formed by the conversion of carbohydrates during storage. Meera (2019) reported TSS content of 8 ^{o}Brix in avocado fruit spread added with KMS (0.25 g), citric acid (0.1 g) and sugar (100 g).

Bishnoi *et al*. (2016) reported increase in TSS of strawberry fruit pulp during storage, which was most preserved in sodium benzoate at 500 ppm under low temperature storage.

2.4.1.8. Titratable acidity

Acidity influences the flavour, antimicrobial activity and the deterioration of the sample. Meera (2019) determined the acidity of avocado fruit spread added with KMS (0.25 g), citric acid (0.1 g) and sugar (100 g), in which highest value was seen in the

sample stored in polythene bags at ambient temperature (0.026 %) and lowest in glass bottle at refrigerated temperature (0.013 %).

Bishnoi *et al*. (2016) reported increase in acidity of strawberry pulp during storage time, in a faster rate at room temperature than at low temperature, which might be due to the conversion of sugars into acids.

2.4.1.9. Total protein

Proteins are major constituent in fruits, which may not change more under low temperature storage (Sikora *et al*., 2013). Meera (2019) reported protein content of 3.1 g/100g in avocado fruit spread added with KMS, citric acid and sugar in the proportion of 0.25:0.1:100. Jobil *et al*. (2021) formulated avocado fruit juice, with protein content ranged from 1.36 ± 0.32 to 6.13 ± 0.05 g/mL.

2.4.1.10. Vitamin C

Ascorbic acid or vitamin C is a major antioxidant in avocado which protects the mesocarp from discoloration due to oxidative stress (Tesfay, 2009). Pathirana *et al*. (2013) reported that total ascorbic acid content declined in avocado pulp under low temperature and low oxygen storage conditions. Meera (2019) reported that vitamin C content of avocado fruit spread added with KMS (0.25 g), citric acid (0.1 g) and sugar (100 g) was 7.32 mg/100g.

2.4.1.11. Total phenols

Accumulation of phenols in the fruit pulp enhances the antioxidant potential of the tissue and hence improves its quality as well as health benefit (Tesfay, 2009). Meera (2019) reported that total phenolic content of avocado fruit spread added with KMS (0.25 g), citric acid (0.1 g) and sugar (100 g) was 18.40 mg/100g. Ospina *et al.* (2019) reported that main deterioration of avocado pulp was the browning which occurred by the enzymatic reactions of polyphenoloxidase and peroxidase, using phenolic compounds as substrates and for oxidation, respectively.

2.4.1.12. Total carbohydrates

D-mannoheptulose and perseitol are the dominant sugar present in the avocado mesocarp, which have antioxidant potential. Meera (2019) reported carbohydrate content of 11 g/100g in avocado fruit spread added with KMS, citric acid and sugar in the proportion of 0.25:0.1:100. Marín-Obispo *et al*. (2021) reported that carbohydrate content in avocado pulp was inversely related with the lipid content.

2.4.1.13. Total fat

Gatbonton *et al*. (2013) reviewed that the most appropriate and simplest method for the optimization of emerald green colour avocado oil and oleic acid was soxhlet extraction of freeze dried avocado pulp. Meera (2019) reported total fat content of 42 g/100g in avocado fruit spread added with KMS, citric acid and sugar in the proportion of 0.25:0.1:100. Nasr and El-Hamid (2019) mentioned that high lipid content in avocado pulp resulted in oxidation, rancidity and subsequent off-flavour development and loss of quality during storage.

2.4.1.14. Polyphenol oxidase activity

The darkening of the fruit pulp when exposed to the air is related to the degradation of phenolic compounds by polyphenol oxidase enzyme (PPO) that adversely affect the appearance of the product (Salveti, 1997). Mesocarp discoloration or pulp darkening is due to the oxidation of o-diphenols to o-quinones, by PPO to form brown melanin pigments (Pesis *et al*., 2002). Vieites *et al.* (2012) reported a decrease in PPO activity in Fuerte avocado during the storage period at low temperature and higher values were observed for fruits kept at room temperature. Poor physical handling of avocado cause mechanical injuries such as bruises, abrasions and further cellular and tissue damages which hasten browning due to polyphenoloxidase activity (Munhuweyi *et al.,* 2020).

2.4.1.15. Peroxide value

Peroxide value indicates the intermediate hydroperoxide formation which initiates oxidative reactions that lead to off-flavour development due to the primary degradation of autoxidation and are expressed as milliequivalents of oxygen per kilogram of oil. Kirk and Sawyer (1991) reported that peroxide value in between 20 and 40 meq O_2/kg oil in oily products caused rancid taste. The oxidative reactions during storage can be determined by analysing the peroxide value in the fat or oil by considering the aldehyde or n-hexanal content of the food (Owusu, 2012). Pathirana *et al*. (2013) reported peroxide values in the range of 1.66 to 7.33 meq O_2/kg oil of fresh avocado pulp and detected off-flavours at 4.8 and 7.3 meq O_2/kg oil. It was reported that slow increase in the peroxide value at 5 ºC and rapid increase at 20 ºC during 3 weeks of storage. Jorge *et al*. (2015) reported that highest peroxide index of 5.54 meq O_2/kg in Hass oil, where the international standard for crude, cold-pressed avocado oil, was 15.0 meq O_2 active/kg oil (Codex, 2019). Ortega *et al*. (2013) reported a peroxide value of 3.79 meq O_2/kg in the solvent extracted oil of Hass variety. Meera (2019)

studied the peroxide content of avocado fruit spread added with KMS, citric acid and sugar and the highest value was seen in the samples stored in polyethylene bags at ambient and refrigerated conditions (19.26 meq O_2/kg) while lowest value in glass bottles at refrigerated condition (10.13 meq O_2/kg).

2.4.1.16. Water activity

Water activity influences the mechanical properties as well as safety of the products since water activity in the range of 0.35 to 0.50 resulted in organoleptically unacceptable products while those with water activity of 0.60 would be microbiologically and biochemically stable. Low water activity indicates the low availability of free water for biochemical reactions as well as microbial growth (Dantas *et al*., 2018).

2.4.1.17. Viscosity

Rheological properties depend on the resistance of the fluid and its behaviour during flow which varies according to the temperature and composition of the material. Factors such as extraction, age, storage, degree of saturation and degree of oxidation affect viscosity of the sample. Lower value of viscosity indicated the higher levels of unsaturation (Ikhuoria and Maliki, 2007). Kim *et al*. (2010) reported that fatty acids with more double bonds or unsaturation have less rigid or fixed structure so that it became less tightly packed and less viscous. In a study conducted by Jorge *et al*. (2015) in avocado pulp, reported that the viscosity decreased as temperature increased which might be due to the weakening of intermolecular forces resulted in faster agitation and easier movement and flow. Freezing process reduced the mobility of liquid molecules and increased the viscosity of the interstitial fluid (Celli *et al*., 2016).

2.4.1.18. Microbial load

Microbial growth in a food depends on the intrinsic factors as the composition of food and extrinsic factors like its environment. In food rich in fats and oils, degradation of fats formed short-chain fatty acids resulted in rancid and unpleasant nature (Owusu, 2012). There have been avocado and avocado product associated outbreaks and recalls which were mainly due to the potential contamination with *Salmonella and Listeria monocytogenes*. In the study conducted by Meera (2019) in avocado fruit spread added with KMS, citric acid and sugar, bacterial colonies were present in all samples while yeast and fungal colonies were absent in sample stored in glass bottle under refrigerated condition.

2.4.1.19. Organoleptic evaluation

Organoleptic quality is associated to the consumers' sensory perception and acceptance for ingesting a product which depends on the smell, taste, texture and visual aspects (Ramírez-Gil *et al*., 2019). Pathirana *et al*. (2013) mentioned that formation of hydroperoxide and further oxidation of oil caused rancidity and developed sour and rancid flavours of avocado pulp during storage.

2.4.2. Optimization of process conditions for preparation of avocado fruit powder

2.4.2.1. Avocado fruit powder

In freeze drying, the change of state occurred during sublimation of water minimizes the loss of nutrients from the cell, it does not affect the nutritional properties of the products (Castaneda-Saucedo *et al*., 2014). Nair (2018) reported that yield of freeze dried avocado powder was 4g from 100g of fruit pulp. Salazar *et al*. (2018) observed that freeze drying increased the brightness (L*) of mango fruit powder.

2.4.2.2. Maltodextrin

Maltodextrin is a tasteless, odourless compound that considered as generally recognized as safe (GRAS) which is used as a drying agent as well as binding agent of flavour and fat. It increases solubility in water, bulking and film formation properties, reduces oxygen permeability of wall matrix and minimizes the stickiness of particles during freeze drying (Sansone *et al*., 2011; Caliskan and Dirim, 2015). The physical and chemical properties of maltodextrin had the potential to preserve nutrients in the powdered mixtures especially during the drying processes, independent to the conditions (Shishir and Chen, 2017). It consists of D-glucose units linked by α (1 $\rightarrow$ 4) glycosidic bond, formed by starch hydrolysis (Muzaffar *et al*., 2018).

Jaya *et al*. (2006) mentioned that maltodetrin enhanced the non-stickiness and free flowing properties to the avocado fruit powder. Dantas *et al*. (2018) reported that higher maltodextrin content in avocado samples enhanced the brightness of sample and decreased green and yellow colour. Bio protective osmolytes such as maltodextin and other sugar molecules protected the components like protein from temperature and oxidation by keeping away from the surface. Maltodextrin retained the sensory qualities of fresh avocado fruit tissue with good potential for mass transfer and water activity reduction (Giannakourou *et al*., 2020).

2.4.2.3. Ascorbic acid

Being a potential antioxidant, Olaeta and Roja (1987) used 0.8 % ascorbic acid in avocado fruit slices and pulp to prevent the enzymatic browning. Jaya *et al*. (2006) mentioned that the presence of organic acids imparted stickiness to the fruit powder due to high hygroscopic nature in amorphous state and lost free flowing property at high moisture content.

2.4.2.4. Tricalcium phosphate

Tricalcium phosphate improves flowability of high fat containing powders. James (1971) used 0.15 % tricalcium phosphate along with 25 to 40 % sucrose to produce desired free flowing guava and pineapple powder using vacuum drying. Jaya *et al*. (2006) elucidated the use of tricalcium phosphate as food grade anticaking agent to improve flowability and to inhibit caking tendency by increasing the sticky point temperature.

2.4.2.5. EDTA

EDTA preserved better initial avocado pulp lightness than ascorbic acid by preventing PPO enzymatic browning reactions due to the antioxidant property by chelating metals such as copper (Soliva *et al*., 2000). Guiamba and Svanberg (2016) mentioned the chelating effect of EDTA on inhibiting the enzymatic activity of PPO and protecting ascorbic acid from oxidative degradation to dehydroascorbic acid in mango puree. Ospina *et al*. (2019) mentioned EDTA as one of the commonly used preservatives to control the pulp browning in avocado puree.

2.4.2.6. Potassium sorbate

Sorbic acid is tasteless, sparingly water soluble preservative, effective even in weak acidic medium. Potassium sorbate is the very freely soluble salt of sorbic acid.

Fernandes *et al*. (2010) reported that avocado puree added with antibrowning (ascorbic acid and EDTA) and antimicrobial agents (potassium sorbate) preserved the shelf life and reduced PPO activity and browning rate. Careli-Gondim *et al.* (2019) reported the fungistatic effect of potassium sorbate by inhibiting dehydrogenase enzyme reactions.

2.4.2.7. Freeze drying

Freeze drying/lyophilisation is a process in which water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying) (Gaidhani, *et al*., 2016). It is an effective way for drying

thermolabile substances or substances unstable in aqueous solutions for prolonged storage periods without any adverse effects.

Freeze-drying is a dehydration process, working with the principle of lyophilisation which separates water by sublimation under very low temperature (-40 to -80 ^{o}C) and vacuum conditions. As an emerging alternative of food preservation, freeze-drying maximizes the conservation of taste compounds, non-volatile flavour compounds, volatile constituents in essential oils and protects thermolabile compounds like phenolics, sugars, tocopherols, chlorophylls and lycopene as well as the antioxidant activity while sometimes increased the content of trace (Cu, Fe, Mn and Zn) and minor (B, Na, Mg, P, K, Ca, Ni, Ge, Se) elements due to the reduction of water levels in the product (Castaneda-Saucedo *et al*., 2014).

2.4.2.8. Bulk density

Bulk density is the property of the product obtained after milling or drying, defined by the mass of solid particles, surface moisture and pores occupied in a unit volume. Products with high bulk density are easy to store in small containers when compared to low bulk density products (Quispe-Condori, 2011). Bulk density indicates the volume required to occupy the powder which is important regarding the packaging and transporting facilities and cost. Lower bulk density is related with the higher air occluded which would result in higher oxidation and reduced storage stability (Dantas *et al*., 2018).

Nair (2018) reported the bulk density of freeze dried avocado fruit powder as 0.285 kg/cm^2. Pomegrante juice powder containing maltodextrin had high moisture content and high bulk density attributed to its bulking ability (Adetoro *et al*., 2020).

2.4.2.9. Solubility

Solubility indicates the time required for the powder to reconstitute or dissolve in the solvent. Higher solubility is more desired as it is easier to be reconstituted which would be more beneficial if the powder is used as an additive in a food product. Lower moisture content in the powder leads to crust formation during drying without any shrinkage which makes it easier to be dissolved in a solvent with shorter reconstitution time (Alissa *et al*., 2020). Solubility is an important characteristic of a powder indicating its wettability and dispersibility in solutions. Adetoro *et al*. (2020) observed higher solubility of 96.50 % in powder added with maltodextrin, which imparted crystalline nature to the powder.

2.4.2.10. Hygroscopicity

Hygroscopicity is the property of a powder indicating the rate at which powder absorbs molecules of water from the surrounding atmosphere ascribed to its hydrophilic nature and determined its moisture content. Pomegrante juice powder containing maltodextrin had higher hygroscopicity of 10.20 % (Adetoro *et al*., 2020).

2.4.2.11. Colour value

Colour is an important attribute for visual acceptance of the product. Hunter colour values L*, a*, and b* were used as the indices for measuring the colour of fruit powder. L* values indicates the luminosity or brightness or lightness which was in the range of darkness to lightness (0–100). a* is a coordinate extending from −120 to +120 representing greenness and redness respectively. While b* values represent with negative values for blueness and positive values for yellowness.

The luminosity values for avocado fruit pulp stored at room temperature and refrigeration ranged from 88.5 to 80.8 and 84.5 to 88.8, respectively. At room temperature less negative a* values were observed which indicated the decrease of green colour in the fruit pulp. The yellow intensity, b* values were positive which indicated the presence of yellow component in the fruit pulp (Vieites *et al.*, 2012). Castaneda-Saucedo *et al*. (2014) reported bright green colour to the freeze dried avocado pulp, indicated the presence of chlorophyll and absence of enzymatic or non-enzymatic browning. Although in thawing at room temperature chlorophyll started to degrade and formed pheophytin resulted in browning. Green colour of avocado was represented by a* value in the range of -6.5 to -3.7 (Dantas *et al*., 2018). Mujaffar and Dipnarine (2020) reported freeze dried avocado with green colour similar to fresh avocado puree with L*, a* and b* values of 82.93,-14.32 and 60.38, respectively.

2.4.2.12. Total Soluble Solids

Mujaffar and Dipnarine (2020) reported the total soluble solids of freeze dried avocado powder as 1.93 ± 0.03 ^{o}Brix and remained stable over 12 weeks of storage. Dilrukshi and Senarath (2021) reported that instant green smoothie freeze dried avocado powder added with maltodextrin reduced aggregation and increased TSS from 8-12 % to 21-24 %.

2.4.2.13. Titratable acidity

Nair (2018) reported 5.95 % of acidity in freeze dried avocado fruit powder from cultivar Purple Hybrid collected from Ambalavayal in Wayanad district in the

first month of storage and it raised to 6.06 % in the second month and 6.20 % in third month. It was mentioned that acidity of the product influenced the spoilage rate.

2.4.2.14. Total protein

Proteins have low thermal stability, which may collapse at higher temperature but during freeze drying the rate of cold denaturation is too slow due to the increase in hydrophobic effects (Oyinloye and Yoon, 2020).

Burdon *et al*. (2007) reported that the protein content in freeze dried avocado fruit powder was in the range of 1.50 to 4.50 g/100g. Nair (2018) reported about 3.80 g/100g of protein in freeze dried avocado fruit powder from cultivar Purple Hybrid.

2.4.2.15. Vitamin C

Ceballos *et al*. (2012) reported that in freeze dried soursop with maltodextrin, higher ascorbic acid content was observed in slower freezing rates. As a water soluble vitamin with antioxidant potential, vitamin C is an important nutrient, which amounts to about 22.80 mg/100g in freeze dried avocado fruit powder obtained from cultivar Purple Hybrid collected from Ambalavayal in Wayanad district (Nair, 2018). Marques (2009) mentioned vitamin C content in the range of 31.53 to 32.63 mg/100g in freeze dried avocado powder of Hass variety.

2.4.2.16. Total phenols

Nair (2018) reported that the total phenolic content in freeze dried avocado fruit powder from cultivar Purple Hybrid was 7.66 mg/100g. Total phenols in freeze dried avocado powder obtained from varieties Pollock, Kallar Round, Purple Hybrid and Fuerte were reported as 51.73, 49.04, 48.00 and 46.4 mg/100g in the study conducted by Archana (2019).

Hamid *et al*. (2017) observed slower rate of loss of phenols in refrigerated mulberry drink than ambient storage and more retention in glass bottles than PET, which might be due to slower rate of reactions and absorption of heat to the product.

2.4.2.17. Total carbohydrates

Nayak *et al*. (2008) mentioned that cell collapse during freeze drying resulted in the increase of carbohydrates in the powder. Nair (2018) reported 4.50 g/100g of carbohydrates in freeze dried avocado fruit powder from cultivar Purple Hybrid. Oyinloye and Yoon (2020) reported that carbohydrates vitrified easily during freeze drying and became susceptible to enzymatic reactions, but it remained as porous particle which determined the bulk density and compressibility of the powder.

2.4.2.18. Total fat

Nair (2018) reported 53.00 g/100g of fat content in freeze dried avocado fruit powder from cultivar Purple Hybrid. Castaneda-Saucedo *et al*. (2014) mentioned that freeze dried avocado powder maintained porous light weight structure without any compaction and subsequent oil exudation. It was reported that freeze drying lowered linoleic fatty acid but it minimized the autooxidation of fat and maintained the colour of powder without any enzymatic browning.

2.4.2.19. Peroxide value

Peroxide value is used to determine the rate of initial degradation by lipid oxidation results in the formation of hydroperoxides. Peroxide value indicating the oxidation of fat and formation of lipid peroxides and hydroperoxides was reported as 12.80 meq O_2/Kg in the first month which increased to 14.20 meq O_2/Kg in freeze dried avocado fruit powder of cultivar Purple Hybrid stored after three months in aluminium foil pouches (Nair, 2018). Cortés-Rodríguez *et al*. (2019) mentioned that oxidation of the avocado fruit tissue began from the initial processing operations such as pulp cutting and mashing by the direct exposure to oxygen and light.

2.4.2.20. Water activity

Udara *et al*. (2019) reported that freezing reduced the water activity and thereby controlled microbial activities by reducing the available free water and enzymatic activities by denaturing the globular proteins in enzymes. Moisture content of a powder is associated with its drying efficiency and it further related to the water activity. Lower moisture content can be correlated with the lower water activity which attributed to the storage stability and water activity below 0.87, 0.88 and 0.80 prevent the growth of microorganisms like bacteria, yeasts, and moulds respectively. Lower water activity hindered the agglomeration and caking of the powders (Adetoro *et al*., 2020). Dantes *et al.* (2018) reported that water activity below 0.60 in spray dried avocado powder with microbiological and chemical stability and it became organoleptically unacceptable at 0.35 and 0.50 water activity.

2.4.2.21. Microbial load

Nair (2018) reported that the microbial count was absent in freeze dried avocado fruit powder of cultivar Purple Hybrid stored for 3 months in aluminium foil pouches. Dilrukshi and Senarath (2020) reported that even the unit operations of peeling, cutting and pulping were source of microbial growth, total microbial count was low in freeze

dried avocado smoothie powder as it became unsuitable for survival of microorganisms due to the lower water content.

2.4.2.22. Organoleptic evaluation

Freeze dried powder retains the nutritional and bioactive composition in more concentrated form with higher sensory acceptability and microbial stability, ensuring longer period of storage. Jakubczyk and Jaskulska (2020) compared fresh soup and rehydrated soup of freeze dried vegetable powder and it was observed that rehydrated soup obtained higher scores for all other sensory attributes except colour.

2.5 Preparation of instant fruit shake

Nair (2018) mentioned that fresh avocado milk shake yield high energy with HDL cholesterol, ideal for counteracting undernourished persons. From the sensory evaluation of fresh avocado milk shake with different proportions of fresh avocado pulp and equal quantity of milk and sugar, the highest mean scores for appearance (41.50), colour (38.05), flavour (37.90), texture (41.15), taste (40.80) and overall acceptability (34.40) were obtained in treatments with highest quantity of avocado pulp.

Nair (2018) reported that among the sensory evaluation of avocado powder milk shake with different proportions of freeze dried avocado powder and equal quantity of milk and sugar, the highest mean score for appearance (41.35), colour (41.65), flavour (40.05), texture (40.90), taste (40.85) and overall acceptability (42.20) were obtained in treatments with least quantity of avocado powder.

2.6. Cost analysis

Cost analysis is used to determine the expenses require to develop a product and to sell it which includes purchase of raw materials, processing, packaging, storing, transporting and marketing charges. It helps to predict whether the product would be profit or loss. Economic feasibility has primary importance while developing a product which is based on the affordable prices and cost effectiveness (Meera, 2019).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study was undertaken in the Department of Post Harvest Technology of the College of Agriculture, Vellanikkara under Kerala Agricultural University, during 2018-2021. Fresh and mature avocado fruits of 27 accessions were collected from Ambalavayal in Wayanad district, located at 974 m above MSL and from Kanthalloor in Idukki district, located at 1525 m above MSL and one accession from Thrissur district (plains), located at 21 m above MSL, in Kerala. Accessions 1 to 14 were collected from Wayanad and accessions 15 to 26 from Idukki district and accession 27 from Thrissur. The fruits were analysed for twenty five horticultural traits according to 'Descriptors for Avocado' given by IPGRI (1995) and the biochemical and nutritional attributes were also quantified.

3.1. Characterisation of avocado accessions

Characterisation of avocado accessions was done according to 'Descriptors for Avocado (*Persea* spp.)' by International Plant Genetic Resources Institute (IPGRI, 1995) an autonomous international scientific organization operating under the aegis of the Consultative Group on International Agricultural Research (CGIAR). Twenty five horticultural traits were classified as given below (Plate 1).

3.1.1. Horticultural traits

3.1.1.1. Fruit shape

Avocado fruit shape was classified as oblate, spheroid, high spheroid, ellipsoid, narrowly obovate, obovate, pyriform, clavate and rhomboidal.

3.1.1.2. Fruit length [cm]

Fruit length was measured as average of five fruits using meter scale.

3.1.1.3. Fruit diameter [cm]

Fruit diameter was measured at the broadest part in average of five fruits using meter scale.

3.1.1.4. Fruit size uniformity

Fruit size uniformity was classified as low, intermediate and high among the fruits.

3.1.1.5. Fruit weight

Fruit weight was measured as average of five fruits and expressed in gram.

3.1.1.6. Fruit base shape

Fruit base shape was classified as depressed, flattened, inflated and pointed.

3.1.1.7. Fruit apex shape

Fruit apex shape was characterized as deeply depressed, slightly depressed, flattened, rounded and pointed.

3.1.1.8. Fruit apex position

Fruit apex position was observed as either in central or asymmetrical.

3.1.1.9. Ridges on fruit

Ridges on fruit was observed as none (absent), partial and entire on the fruit surface.

3.1.1.10. Gloss of fruit skin

Gloss of fruit skin was observed in the range of weak, medium and strong.

3.1.1.11. Pedicel position on fruit

Pedicel on fruit was observed in central, asymmetrical, very asymmetrical and extremely asymmetrical in position.

3.1.1.12. Fruit skin surface

Smooth, intermediate and rough fruit skin surface were observed generally in avocado fruits.

3.1.1.13. Fruit skin colour

Fruit skin colour of ripe fruits was recorded with assistance of Royal Horticultural Society (RHS) colour chart and was characterized as light green (green group 142A), green (green group 141B), dark green (green group 135A), yellow (yellow-green group 154A), red (orange-red group 30C), purple (purple group 79C), black (black group 202A) and speckled.

3.1.1.14. Fruit skin thickness (Average of five observations per accession)

Fruit skin thickness of average of five observations per accession was measured using vernier calliper and it was noted in the range of 1 to 3 mm.

3.1.1.15. Pliability of fruit skin

Avocado fruit skin thickness were characterized as either pliable or brittle.

3.1.1.16. Adherence of skin to flesh

Adherence of skin to flesh was classified as slight, intermediate and strong.

3.1.1.17. Colour of flesh next to skin

Colour of flesh next to skin of ripe fruits was recorded with assistance of Royal Horticultural Society (RHS) colour chart and was characterized as ivory (yellow group 4D), light yellow (yellow-green group 154D), yellow (yellow group 6B), deep yellow (yellow group 7A), light green (yellow-green group 145A) and green (yellow-green group 144A) .

3.1.1.18. Colour of flesh next to seed

Colour of flesh next to seed was recorded with assistance of Royal Horticultural Society (RHS) colour chart and was characterized as ivory (yellow group 4D), light yellow (yellow-green group 154D), yellow (yellow group 6B), deep yellow (yellow group 7A), light green (yellow-green group 145A) and green (yellow-green group 144A).

3.1.1.19. Flesh texture

Flesh texture was characterized as watery, buttery, pastose (doughy) and granular.

3.1.1.20. Sweetness of flesh

Fruit flesh was characterized with low, intermediate and high sweetness.

3.1.1.21. Bitterness of flesh

Fruit flesh was characterized with low, intermediate and high bitterness.

3.1.1.22. Fibre in flesh

Presence of fibre in tasted flesh of ripe fruits was classified as low, intermediate and high.

3.1.1.23. General taste of flesh

General taste of flesh of ripe avocado fruits was characterized as very poor, poor, fair, good and excellent.

3.1.1.24. Degree of discolouration of open fruit after 4 h

Degree of discolouration of cut opened fruit after 4 h was observed in the range of low, intermediate and high.

3.1.1.25. Colour of discolouration

Fruit flesh discoloration of cut opened fruits were characterized as blue, brown, grey and black.

3.1.2. Biochemical characterisation

3.1.2.1 Total soluble solids

Initially, the refractometer is tested for accuracy with the help of distilled water, by looking through the eyepiece with the projection inlet facing towards the light. Brix reading is taken where the light and dark areas meet on the scale. Total soluble solids of avocado fruit samples were measured using a digital refractometer (ATAGO, PAL-1, 0-53 ºBrix, Japan) and were expressed in ºBrix (Plate 2).

3.1.2.2 Titratable acidity

5 g of fruit sample was weighed and ground using pestle and mortar and made up to 100 mL in volumetric flask using distilled water. 10 mL of the filtered aliquot taken in a conical flask was added with 2-3 drops of phenolphthalein and titrated against 0.1 N sodiumhydroxide. Appearance of light pink colour was denoted as the end point. The value was expressed in per cent of malic acid which is the predominant acid present in the avocado fruit (AOAC 1998). . Acidity was calculated by the following equation,

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

3.1.2.3 Vitamin C

5 g of the sample was ground and made up to 100 mL with 3 % metaphosphoric acid. 10 mL of filtered aliquot was titrated against 2, 6- dichlorophenol indophenol dye until the end point of faint pink colour appeared (AOAC 1998).

$$\text{Dye factor} = \frac{\text{mg of L-ascorbic acid}}{\text{mL of dye consumed}}$$

Plate 1. Horticultural traits of avocado fruit

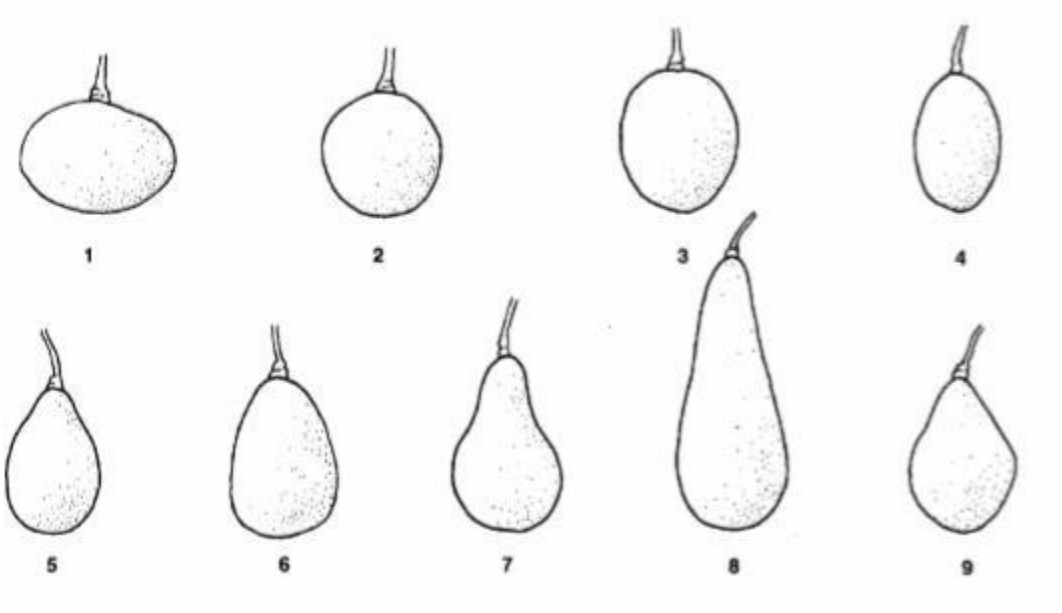


Plate 1a. Fruit shape

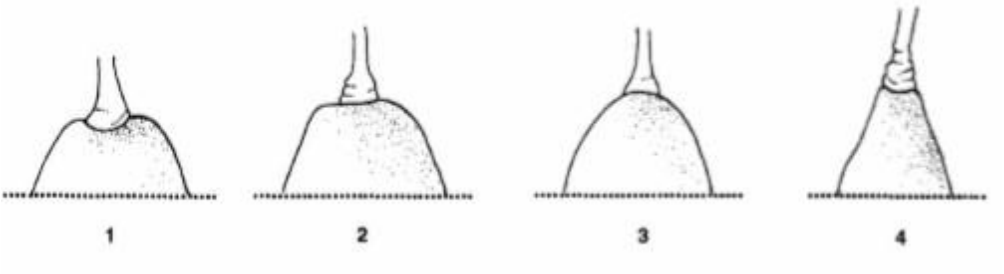


Plate 1b. Fruit base shape

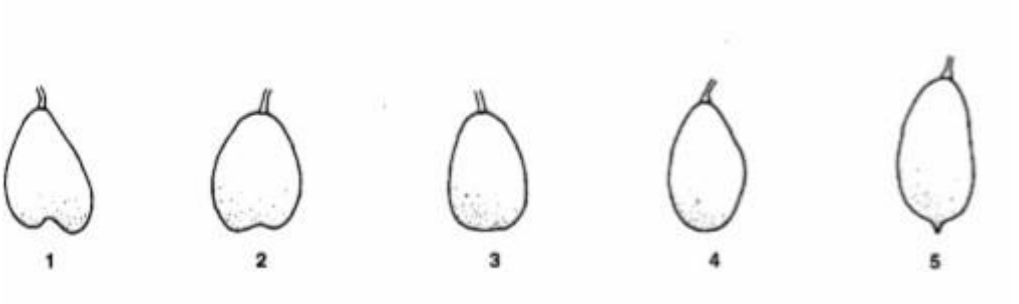


Plate 1c. Fruit apex shape

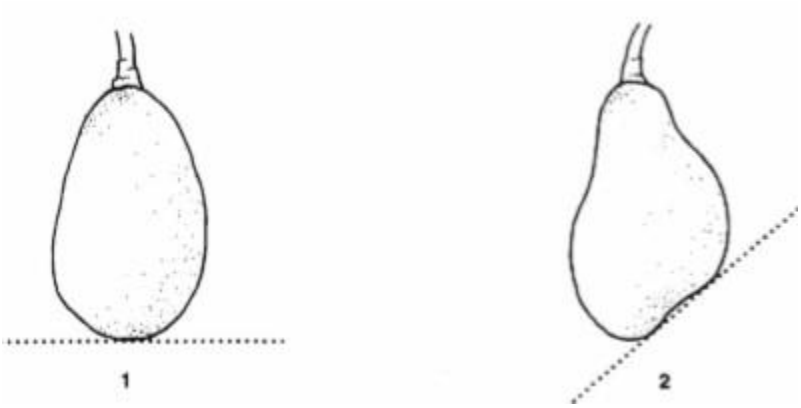


Plate 1d. Fruit apex position

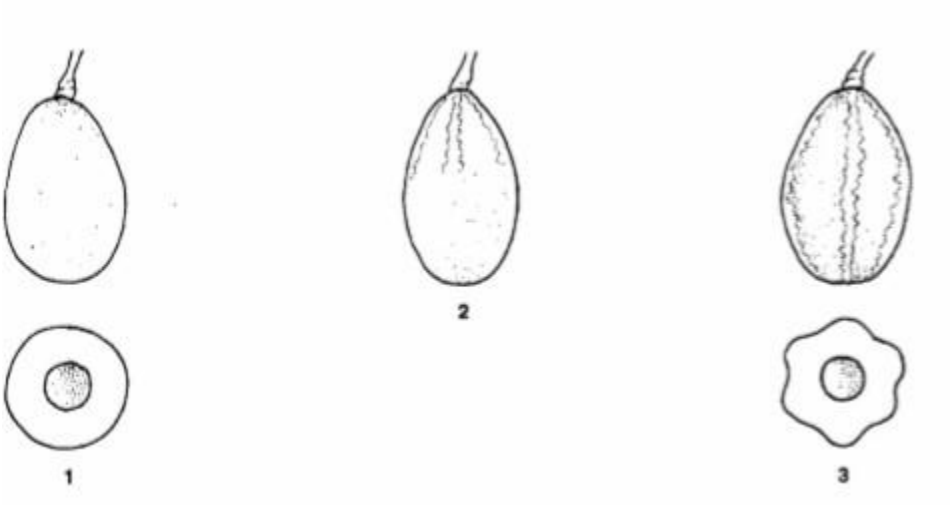


Plate 1e. Ridges on fruit

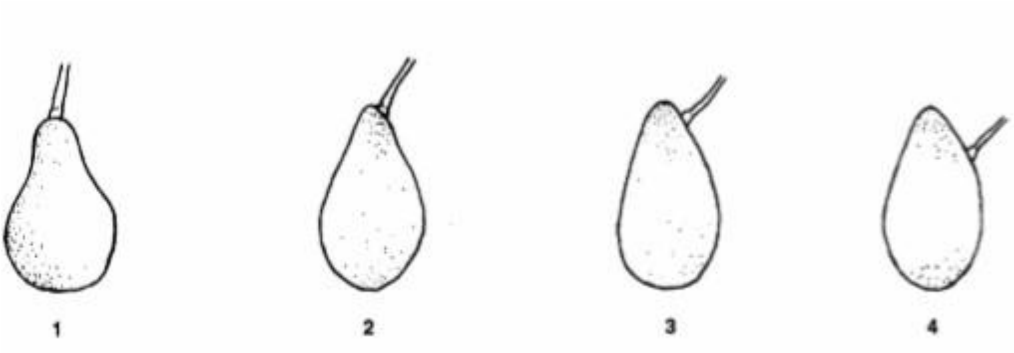


Plate 1f. Pedicel position on fruit

$$\text{Vitamin C (mg/100g)} = \frac{\text{Dye factor x Titre value x Volume made up x 100}}{\text{Weight of sample x Volume of aliquot}}$$

3.1.2.4 Total carbohydrates

100 mg of sample taken in a boiling tube was hydrolysed in a boiling water bath for ++three hours with 5mL of 2.5 N hydrochloric acid and cool to room temperature. The dried sample was neutralised with solid sodium carbonate until the effervescence ceased and made up the volume to 100 mL. 0.5 mL of aliquots was taken for analysis from the supernatant of the centrifuged sample. Standards were prepared from 0.2 to 1 mL of the working standard along with blank and made up to 1 mL with distilled water. A green coloured compound was formed with the addition of 4 mL of anthrone reagent which was further heated for eight minutes in a boiling water bath. After rapid cooling, absorbance was taken at 630 nm with spectrophotometer (UV-Visible 1800 spectrophotometer, Shimadzu, Kyoto, Japan) (Plate 2). From the standard graph plotting concentration on X-axis versus absorbance on Y-axis, the amount of carbohydrate was calculated and expressed as gram per 100g (Hewit, 1958).

3.1.2.5 Total protein

The amount of protein in the sample was determined according to procedure suggested by Lowry *et al*. (1951). 500 mg of sample was extracted with 5-10 mL Tris-HCl buffer and centrifuged. Along with the blank and standards from 0.2 to 1 mL, supernatant of sample extract (0.1 mL) were added in test tubes and made up each to 1 mL. 5 mL of alkaline copper sulphate solution was added, mixed well and kept for 10 minutes at room temperature, after which 0.5 mL of Folin-Ciocalteau reagent was mixed thoroughly and kept for 30 minutes in dark. The amount of total protein in the sample was determined from calibration curve with mg of protein on X- axis and absorbance measured at 660 nm on Y-axis and expressed in g/100g of sample.

3.1.2.6 Total phenols

Sample (1 g) was extracted repeatedly twice with 80 % ethanol and centrifuged at 10,000 rpm for 20 minutes to homogenise, pooled the supernatant and evaporated to dryness. Three minutes after adding with Folin-Ciocalteau reagent (0.5 mL) to the extract, 2 mL of 20 % sodium carbonate was mixed thoroughly and kept at room temperature for 1 hour. The absorbance was measured with spectrophotometer at 650

nm. Total phenol content was calculated from the standard curve and expressed as milligram per 100g of sample (Asami *et al*. 2003).

3.1.2.7 Total flavonoids

Total flavonoid content was determined according to the procedure suggested by Dewanto *et al*. (2002). About 1 mg of sample was dissolved and made up to 5 mL with distilled water in a 10 mL volumetric flask. It was added with 0.3 mL of sodium nitrite (5 %), followed by 0.3 mL of aluminium chloride (10 %) after 5 minutes and 2 mL of 1 M sodium hydroxide after 6 minutes. Mixed the solution thoroughly and made up the volume to 10 mL with distilled water and allowed to stand for 30 minutes. Total flavonoid content was determined as the catechol equivalent (mg/100g) using the calibration curve of catechol standard solutions with absorbance measured at 510 nm.

3.1.2.8 Total fat

Total fat was extracted from a known quantity of sample with petroleum ether. It was then distilled off completely in soxhlet apparatus (Plate 2), dried and the fat content was determined from the dry weight of sample (Ranganna, 1986).

$$\text{Total fat (\%)} = \frac{\text{Weight of the fat (g)}}{\text{Weight of sample (g)}} \text{ X } 100$$

3.1.2.9 Oleic acid

1-10 g of oil or melted fat was dissolved in 50 mL of the neutral solvent (25 mL ether mixed with 25 mL 95 % alcohol) in a 250 mL conical flask and a few drops of phenolphthalein were added to it. The content was titrated against 0.1 N KOH with constant shaking until a pink colour persisting for 15 seconds developed (AOAC, 2000).

$$\text{Oleic acid (mg KOH/g)} = \frac{\text{Titre value x Normality of KOH x 28.2}}{\text{Weight of the sample (g)}}$$

3.1.2.10 Total ash

10 gram of sample powder was placed in a pre-weighed silica crucible (W_1). The crucible was heated at 550-600 ºC in a muffle furnace for about 2-3 hours. The crucible was cooled in a desiccator and weighed (W_2). The crucible was heated again in the furnace for half an hour, cooled and weighed in order to ensure complete ashing.

This was repeated consequently till the weight of ash became constant (ash became greyish white) (Ranganna 1986). Total ash content was calculated using the formula:

$$\text{Total ash content (\%)} = \frac{[W_2 - W_1]}{\text{Weight of the sample}} \text{ X } 100$$

3.1.2.11 Calcium and potassium

Minerals such as calcium and potassium were estimated in flame photometric method (Flame photometer CL 378, Elico Ltd, India) (Plate 2) using the ash solution prepared from the dry ash of the samples by dissolving with 1 mL hydrochloride dissolve in 100 mL water. The solution was allowed to evaporate in water bath for 1 hour and after cooling, filtered it through Watman No. 1 filter paper, and was made up to 100 mL. The prepared ash solution can be used for the analysis of minerals like calcium, potassium and iron (Ranganna, 1986). Calcium and potassium content of samples were measured with working standards at 10, 20, 50 and 100 ppm concentration prepared from 624 mg calcium carbonate and 477 mg potassium chloride dissolved in 250 mL double distilled water as stock solution, respectively.

3.1.2.12 Iron

Ash solution prepared by dry ashing the sample was used for the estimation of iron content. 5 mL of ash solution was added with 0.5 mL of concentrated sulphuric acid, 1 mL potassium persulphate and 2 mL potassium thiocyanate and made up the solution to 15 mL with distilled water and measured the developed colour at 480nm (Ranganna, 1986). Iron content was measured using the formula,

$$\text{Iron (mg/100g)} = \frac{\text{OD of sample x 0.1 x total volume of ash solution x 100}}{\text{OD of standard x 5 x weight of sample taken for ashing}}$$

3.1.2.13 Crude fibre

Sample was extracted with petroleum ether and dried. 2 g of the dried material was boiled with 200 mL of concentrated sulphuric acid for 30 minutes and filtered through muslin cloth and washed with boiling water until washings were no longer acidic. Then the extract was again boiled with 200 mL of sodium hydroxide solution for 30 minutes and filtered through muslin cloth and washed with 25 mL of boiling 1.25 % sulphuric acid, three 50 mL portions of water and 25 mL of alcohol. The residue was dried for 2 hours at 130±2 °C in pre weighed ashing dish (W_1) and weighed the dish

after cooling in a desiccator (W_2). The remnants were ignited for 30 minutes at 600±15 °C and reweighed the dish after cooling in a desiccator (W_3) (Ranganna, 1986).

$$\text{Crude fibre (\%)} = \frac{\text{Loss in weight on ignition } (W_2\text{-}W_1)\text{-}(W_3\text{-}W_1)}{\text{Weight of the sample}} \text{ X } 100$$

3.1.2.14. Antioxidant activity of superior avocado accession (Acc. 25)

3.1.2.14.1. Antioxidant activity by DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of methanolic extracts of the sample was determined according to the procedure reported by Brand-Williams *et al*. (1995) with slight modification. The stock solution was prepared by dissolving 4 mg DPPH in 100 mL methanol and kept in refrigerator for subsequent use.

DPPH stock solution diluted with methanol to an absorbance of about 0.98 at 517 nm, was used as the working solution. Various concentrations (50- 300 ppm) of sample extract (1 mg/mL methanol) along with control were made up to 5 mL with methanol, from which 0.5 mL was pipetted out and added with 2.5 mL DPPH reagent. The reaction mixture was kept in dark at room temperature for 30 minutes and the absorbance was measured at 517 nm. The antioxidant activity was indicated by the disappearance of the purple colour of DPPH to yellow in test samples. Ascorbic acid was used as the standard. The percent antioxidant activity or radical scavenging activity was calculated using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \text{ X } 100$$

where, Ac and As are the absorbance of control and sample respectively.

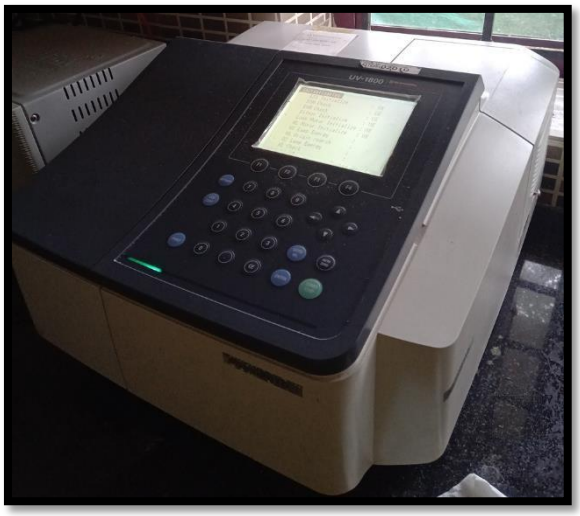
3.1.2.14.2. Antioxidant activity by FRAP Assay

Total antioxidant activity was determined by ferric reducing antioxidant power (FRAP) assay depending on the procedure described by Benzie and Strain (1996) with some modification in which the FRAP reagent was prepared by mixing 300 mM acetate buffer, 10 mL TPTZ in 40 mM hydrogen chloride and 20 mM ferric chloride in 10:1:1 proportion and incubated for 15 minutes at 37 °C. From various concentrations of plant sample (50-300 ppm) made up with methanol, 150 µL was mixed with 2.85 mL of FRAP reagent along with a control (3 mL of FRAP reagent). After incubating the

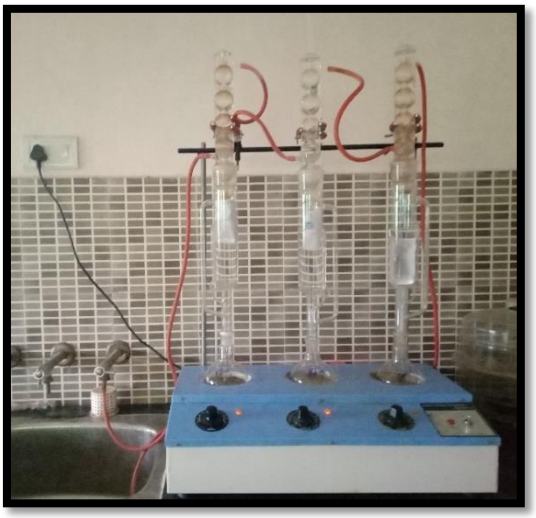
Plate 2. Equipments used for biochemical analysis of avocado fruits

Refractometer

Flame photometer



Spectrophotometer



Soxhlet apparatus

mixture for 30 minutes at 37°C in dark, absorbance was measured at 593 nm. Ascorbic acid was used as the standard. The percentage antioxidant activity was observed using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{(Ac - As)}{Ac} \text{ X } 100$$

where, Ac and As are the absorbance of control and sample, respectively.

3.1.2.14.3. Antioxidant activity by ABTS assay

ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) reagent was prepared by mixing 7 mM of ABTS with 2.45 mM ammonium persulphate in phosphate buffer with pH 7.4 and the reagent was kept in dark at room temperature for 12-16 hours before use. The solution was diluted to an absorbance of 0.7±0.05 with methanol at 734 nm. Methanolic extract of the sample was diluted to various concentrations of 50-800 ppm, from which 10 µL of extract was added with 200 µL of ABTS reagent along with control (Vargas-Ortiz *et al*., 2016). Ascorbic acid was used as the standard. The absorbance was taken at 734 nm and percentage antioxidant activity was observed using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{(Ac - As)}{Ac} \text{ X } 100$$

where, Ac and As are the absorbance of control and sample, respectively.

3.2. Effect of shrink packaging and storage temperature on quality and shelf life of avocado.

Mature avocado fruits of the selected cultivar were surface sanitised with ozonisation (Plate 3) at 2 ppm for 15 minutes, followed by the treatment with 2 % calcium chloride for 30 minutes. The fruits after removal of excess surface moisture were shrink packaged with 25µ polyolefin film. The shrink packaged (Plate 3) fruits were stored under ambient condition (27-32 ºC), refrigerated (4-7 ºC) and also held in a cool chamber (12-13 ºC) at 90% relative humidity to evaluate the quality and shelf life of the fruits. Physical and physiological parameters were observed at 3 days interval and biochemical parameters were taken at initial and final days of storage. Samples were replicated thrice and statistically analysed in completely randomised block design of experiment.

3.2.1. Physical and physiological parameters

3.2.1.1. Shelf life (days)

Shelf life was calculated as the number of days for which one fourth of fruits are affected with decaying, browning and microbial growth. Unmarketability of the fruits was determined based on the visual changes like shrivelling, withering and decaying. Shelf life can be defined as the period at which the predetermined level of quality of the product is maintained under specified storage condition (Perez *et al*., 2004).

3.2.1.2. Physiological Loss in Weight (%) (PLW)

Samples were weighed at the time of storage and up to the end of shelf life at three days interval and the cumulative weight loss was calculated as physiological loss in weight, expressed in percentage.

$$\text{PLW (\%)} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight}} \text{ X } 100$$

3.2.1.3. Respiration rate (% CO_2)

The respiration rate was calculated based on the carbon dioxide produced by the fruit inside the package. The concentration of carbon dioxide (CO_2) in a closed package was measured by taking 1 mL of gas from the headspace and injecting it into a gas analyzer (PBI-Dansensor Checkmate 9900, Ringsted, Denmark). The results displayed were expressed as CO_2 evolved in percentage (Plate 3).

3.2.1.4. Ethylene evolution rate (%)

To determine the ethylene production rate, each avocado fruit was placed in a sealed package for ethylene accumulation under ambient condition for 30 minutes after removal from storage condition. Then, 1 mL of gas was taken from the headspace and injected into an ethylene gas analyzer (PBI-Dansensor Checkmate 9900, Ringsted, Denmark). The results displayed were expressed in percentage (Plate 3).

3.2.1.5. Texture (kg/cm^2)

The Vaiseshika digital pressure tester (Plate 3) was used to determine the firmness of fruits during storage by measuring the pressure necessary to force a plunger of specified size (7.9 to 11.1 mm diameter) into the pulp of fruit. After removing 1/2"-3/4" diameter disc of peel which is required especially for waxy fruits like avocado, the probe was held against the surface of the fruit perpendicularly and forced into the fruit with a steady pressure until the plunger tip penetrated into the pulp up to the notch. The fruits were punched on the middle point of opposite sides of the wider area and at 90^o

Plate 3. Equipments used for pre-treatments and quality analysis of avocado fruits

Ozonizer

Shrink packaging machine

Respiratory gas analyser

Ethylene gas analyser

Fruit pressure analyser

(on each of the opposite cheeks and close to either stem-end or calyx-end) and the average values indicated mean fruit firmness in kg/cm^2.

3.2.1.6. Decay (%)

Fruit decay (rots and disorders) were assessed with the incidence of any visible skin defects such as any fungal or physiological patches and other external rots such as stem end rots, vascular browning, diffused flesh discoloration (DFD) according to New Zealand Avocado Industry Council Fruit Assessment Manual (Dixon, 2003). It was recorded as percentage depending on the presence or absence of decay without any severity score (Burdon *et al*., 2017).

3.2.1.7. Post harvest disease incidence

By visual observations, post harvest disease incidence was detected and the causal organisms were identified by culturing in the Department of Plant Pathology, College of Agriculture, Vellanikkara. Most of the post harvest diseases were due to fungal infections and developing rots causing blemishes to edible parts, reducing appearance and market value that accounted for about 70 % of fruit losses.

3.2.2. Biochemical parameters (initial and final)

3.2.2.1. Total Soluble Solids

Same as described in 3.1.2.1

3.2.2.2. Titratable acidity

Same as described in 3.1.2.2

3.2.2.3. Total carbohydrates

Same as described in 3.1.2.4

3.2.2.4. Total protein

Same as described in 3.1.2.5

3.2.2.5. Vitamin C

Same as described in 3.1.2.3

3.2.2.6. Total phenols

Same as described in 3.1.2.6

3.2.2.7. Total fat

Same as described in 3.1.2.8

3.3. Effect of food additives on quality of frozen avocado during storage

Firm-ripe avocado fruits were cleaned, peeled and cut into longitudinal halves and after removing the seed, the mesocarp was cut into curved equatorial slices of about 70 mm length, 10 mm wide and 3 mm thickness. The fruit slices were immersed in a solution with different proportions of sucrose (20-40 %), along with ascorbic acid (0.5 %) in combination with one of the preservatives (potassium metabisulphite, sodium benzoate, potassium sorbate @ 0.1 %). The treated samples with food additives were quick frozen to -20 ºCin blast freezer (Make: Celfrost D3, Italy) (Plate 4) within 30 minutes, followed by packaging in LDPE pouches with 200 gauge thickness and subsequently stored. The frozen slices were stored at -18 ºCfor three months and evaluated for their quality parameters. Each of the nine treatments were replicated at three times and the results obtained were statistically analysed using Completely Randomised Design (CRD) of experiment.

3.3.1. Total Soluble Solids

Same as described in 3.1.2.1

3.3.2. Titratable acidity

Same as described in 3.1.2.2

3.3.3. Total protein

Same as described in 3.1.2.5

3.3.4. Total phenols

Same as described in 3.1.2.6

3.3.5. Total carbohydrates

Same as described in 3.1.2.4

3.3.6. Total fat

Same as described in 3.1.2.8

3.3.7. Polyphenol oxidase (PPO) activity

PPO enzyme activity was determined by using PPO enzyme extract obtained by homogenizing the avocado fruit pulp with 50 mL of sodium phosphate buffer and centrifuged the filtrate at 12000 rpm at 40 °C for 20 minutes from which the supernatant can be stored at –18 °C for subsequent use.

0.5 mL of the PPO extract was mixed with 0.8 mL of 100 mM sodium phosphate buffer and 0.05 mL of 0.01 M solution of catechol. After incubating the resulting mixture for 30 minutes at 30 °C, 0.8 mL of 2 M perchloric acid was added and placed

in an ice bath. PPO activity was determined by measuring the absorbance in spectrophotometer at 395 nm for 5 minutes. Increase in one unit of absorbance/minute.mL^{-1} of sample indicated one unit of PPO activity and it was expressed in unit/mL (Fujita *et al.*, 1995).

3.3.8. Peroxide value

1 g of avocado oil or fat was weighed and added with 1 g of powdered potassium iodide and 20 mL of solvent mixture in a test tube. Solvent mixture was prepared by mixing two volumes of glacial acetic acid with one volume of chloroform. The liquid was placed in boiling water bath so as to boil within 30 seconds and not more than 30 seconds. It was transferred to a conical flask with 20 mL of 5 % potassium iodide solution and added washings of tube twice each with 25 mL water and titrated against N/500 sodium thiosulphate ($Na_2S_2O_3$) solution until yellow colour almost disappeared. As an indicator 0.5 mL of 1 % starch solution was mixed thoroughly and titrate again till the blue colour just disappeared. A blank was also prepared alongside.

$$\text{Peroxide value (milliequivalent peroxide/kg sample)} = \frac{S \times N \times 100}{\text{Weight of fat (g)}}$$

S = Titre value of $Na_2S_2O_3$ (mL) (Test-blank) and N= normality of $Na_2S_2O_3$

3.3.9. Water activity

Water activity (a_w) is the ratio of partial vapour pressure of water in a food sample to the partial vapour pressure of pure water at a specific temperature. The water activity of the samples were measured using a water activity meter (AquaLab, Pre 40412, Decagon Devices, USA) (Plate 4). After calibration with sodium chloride solution with known water activity, the sample cup was filled to about half of its capacity with avocado fruit samples and a digital output displayed the water activity values of the samples at 25 to 28 ^{o}C. Water activity meter is based on a dew point block method in which, the relative humidity of air in the chamber is recorded at an equilibrium when condensation of water first occurred on a mirror detected in a photoelectric cell, is considered as the water activity of the sample

3.3.10. Enumeration of microbial population

Quantitative assay of microbial load was conducted using serial dilution spread plate technique suggested by Agarwal and Hasija (1986) (Plate 5). Samples (10 g) were mixed thoroughly in 100 mL of sterile distilled water as 10^{-1} dilution. 1 mL aliquot was

pipetted out into test tube with 9 mL sterile distilled water for 10^{-2} dilution. This was continued up to 10^{-5} dilution. Using micropipette one millilitre each from 10^{-3}, 10^{-4}, 10^{-5} dilutions were taken for spread plating fungi, yeast and bacteria cultures to the petridishes, respectively. The growing media used for fungi, yeast and bacteria cultures were Martin Rose Bengal Agar, Sabouraud Dextrose Agar and Nutrient Agar, respectively and the media composition is shown in appendix II. 20 mL of melted and cooled medium was poured into the petridish and swirled it in clockwise and anti-clockwise directions for even distribution. After solidifying the medium, petridish was kept for 5 days, 3 days and 24 hours incubation at room temperature for fungi, yeast and bacteria culture development, respectively. Each sample was replicated thrice in petridishes and colonies counted were expressed as cfu g^{-1} of sample. Number of microorganisms per 10 gram of treated samples were calculated based on the formula: Number of colony forming units (cfu per g of sample) =

$$\frac{\text{Total no. of colony formed x Dilution Factor}}{\text{Aliquot plated}}$$

3.3.11. Organoleptic evaluation

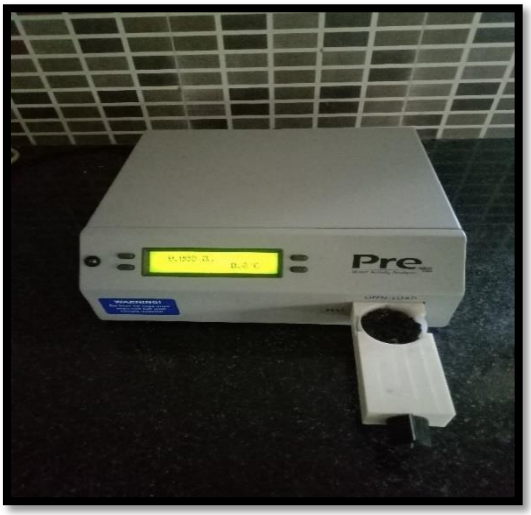
Organoleptic evaluation provides the consumers' sensory perception when ingesting a product with respect to their own smell, taste, texture and visual aspects (Peryam and Pilgrim, 1957) (Plate 5).

Sensory analysis was conducted using 9 point hedonic scale. The samples were evaluated for sensory attributes *viz*. appearance, colour, flavour, odour, texture, taste, after taste and overall acceptability by a semi trained panel consisting of 15 members.

Organoleptic tests were statistically analysed by Kendall's co-efficient of concordance in SPSS software and mean scores were used to differentiate the best storage conditions. Kendall's Coefficient of Concordance (W) is the measurement of degree of association or agreement of rankings while assessing same samples by several judges. It ranges from 0 to 1 in which '0' indicates no agreement and '1' indicates perfect agreement. It implies that higher value of Kendall's indicates stronger association (Field, 2005). The corresponding mean rank for appearance, colour, odour, texture, taste, aftertaste, and overall acceptability were expressed in radar chart.

Plate 4. Equipments used for frozen avocado fruit slices pre-treatment and quality analysis

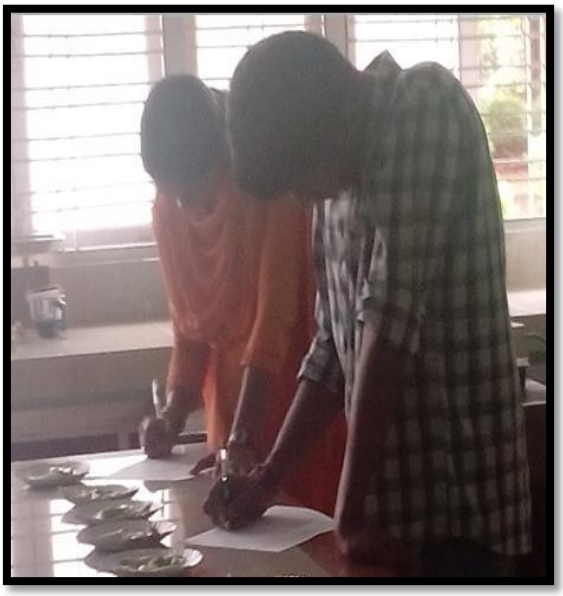
Blast freezer



Water activity meter

Plate 5. Methods for quality analysis of avocado fruit products

Microbial analysis in Laminar Air Flow Chamber



Organoleptic evaluation

3.4. Effect of food additives on quality of avocado pulp and avocado fruit powder

3.4.1. Process standardization for preparation and storage of avocado pulp

Ripe avocado fruits were surface sanitized by ozonisation @ 2 ppm for 15 minutes, followed by peeling, slicing and grinding into pulp. The pulp was added with 0.5 % citric acid and ascorbic acid separately, in combination with a preservative, either potassium metabisulphite (KMS) or sodium benzoate @ 0.1 %. The preserved pulp was enclosed in glass jars and in vacuum packed LDPE (200 gauge) bags separately (Plate 6). The packaged pulp was stored under ambient and refrigerated conditions for 3 months. The observations were recorded at monthly intervals.

3.4.1.1. Pulp yield (%)

Pulp yield was calculated from the weight of pulp obtained from the fruit with known weight, expressed in percentage of weight basis.

$$\text{Percent of pulp} = \frac{\text{Weight of pulp}}{\text{Total weight of fruit}} \times 100$$

3.4.1.2. Total Soluble Solids

Same as described in 3.1.2.1.

3.4.1.3. Titratable acidity

Same as described in 3.1.2.2.

3.4.1.4. Total protein

Same as described in 3.1.2.5.

3.4.1.5. Vitamin C

Same as described in 3.1.2.3.

3.4.1.6. Total phenols

Same as described in 3.1.2.6.

3.4.1.7. Total carbohydrates

Same as described in 3.1.2.4.

3.4.1.8. Total fat

Same as described in 3.1.2.8.

3.4.1.9. Polyphenol oxidase activity

Same as described in 3.3.7.

3.4.1.10. Peroxide value

Same as described in 3.3.8.

3.4.1.11. Water activity

Same as described in 3.3.9.

3.4.1.12. Viscosity

Viscosity is the measurement of a fluid's internal resistance to flow which can be typically designated in units of centipoise or poise. Viscosity of avocado fruit pulp was measured using viscometer (Ametek Brookfield DVE Viscometer, United States) (Plate 6) of low viscosity model with 4 spindles and a narrow guard leg. Spindle was attached to the lower shaft and the sample in a suitable container was placed in the guard leg. Immersing the spindle and allowing to rotate it into the sample by switching "ON" the motor until the reading was stabilized. Viscosity (cP) was calculated by multiplying the reading displayed in the equipment with the factor corresponding to the spindle and speed combination that specified for each viscometer model.

Viscosity in cP (mPa•s) = Dial reading x Factor

Example: For LV Viscometer used with spindle 1 at 6 rpm, the factor was specified as 10 and when reading displayed in the equipment was 75, viscosity would be 75 x 10 = 750 cP.

Viscosity is proportional to the spindle speed in which minimum viscosity were observed with largest spindle at the highest speed and maximum range with smallest spindle at slowest speed.

3.4.1.13. Microbial load

Same as described in 3.3.10.

3.4.1.14. Organoleptic evaluation

Same as described in 3.3.11.

3.4.2. Optimization of process conditions for preparation of avocado fruit powder and preparation of instant fruit shake

Ripe avocado fruits were washed, peeled, sliced and subsequently ground into pulp. This pulp was added with different combinations of maltodextrin (2, 3, 4 and 5 %) along with ascorbic acid (1 %), tricalcium phosphate (0.15 %), ethylenediamine tetra acetic acid (EDTA) (0.05 %) and potassium sorbate (0.05 %). The prepared pulp was freeze dried in SP VirTis Benchtop K model freeze drier (Plate 7) at -70 ºCand 25 milli torque vacuum for 36 hours to obtain freeze dried avocado powder. The dried produce was powdered and sieved. The powder obtained were packed in LDPE laminated aluminium pouches (200 gauge) using continuous band sealer (Plate 7) at 200 ºCand in glass jars and stored under ambient and refrigerated conditions for 3 months. Observations were recorded at monthly intervals.

Plate 6. Equipments used for avocado fruit pulp pre-treatment and quality analysis

Vacuum packaging machine

Brooksfield Viscometer

3.4.2.1. Bulk density

Bulk density was measured from a known quantity avocado fruit powder, passed through a sieve (1 to 2 mm mesh size) so as to break up agglomerates and filled into a graduated cylinder without compacting. Bulk density was calculated by dividing mass of the avocado fruit powder with volume occupied by the same sample. The bulk density is expressed in grams per millilitre (g/mL) although the international unit is kilogram per cubic metre (1 g/mL = 1000 kg/m^3) as the measurements were made using cylinders (WHO, 2012).

3.4.2.2. Solubility

Solubility was determined by the method described by Cano-Chauca *et al*. (2005) with some modifications. 1 g freeze dried avocado fruit powder was mixed in 25 mL of distilled water, stirred thoroughly and centrifuged for 5 min at 1000 rpm. The supernatant was transferred to a pre-weighed petri dish and dried at 105 °C for 5 h. The solubility percentage was calculated by the weight difference.

$$\text{Solubility (\%)} = \frac{\text{Mass of soluble powder}}{\text{Mass of total powder}} \times 100$$

3.4.2.3. Hygroscopicity

About 1 g of freeze dried avocado fruit powder was spread evenly on pre-weighed petri dishes for high surface area contact between humid air and powder. The dishes were placed in desiccators at room temperature. Weight gain due to the moisture sorption was recorded at 2 h intervals until a constant weight was obtained indicating highest amount of moisture absorbed by the powder. The weight increased per gram of powder solids after being subjected to the atmosphere indicated hygroscopicity (Manickavasagan *et al*. 2015). Powder with less than 10% hygroscopicity value is considered as good 'non-hygroscopic' powder (Jaya *et al*., 2006).

$$\text{Hygroscopicity (\%)} = \frac{\text{Gram of moisture absorbed}}{\text{Gram of powder}} \times 100$$

3.4.2.4. Colour values (L, a, b)

The colour properties of the avocado powder were determined using UV1800-2401PC colour analysis spectroscopy software, Shimadzu in terms of L* (light and dark), a* (red and green) and b* value (yellow and blue). L* represents the lightness to darkness (0–100), a* represents red (positive) and green (negative) colour, while b* represents yellow (positive) and blue (negative) colour.

3.4.2.5. Total Soluble Solids

Same as described in 3.1.2.1.

3.4.2.6. Titratable acidity

Same as described in 3.1.2.2

3.4.2.7. Total protein

Same as described in 3.1.2.5.

3.4.2.8. Vitamin C

Same as described in 3.1.2.3.

3.4.2.9. Total phenols

Same as described in 3.1.2.6.

3.4.2.10. Total carbohydrates

Same as described in 3.1.2.4.

3.4.2.11. Total fat

Same as described in 3.1.2.8.

3.4.2.12. Peroxide value

Same as described in 3.3.8.

3.4.2.13. Water activity

Same as described in 3.3.9.

3.4.2.14. Microbial load

Same as described in 3.3.10.

3.4.2.15. Organoleptic evaluation

Same as described in 3.3.11.

Plate 7. Equipments used for the preparation of freeze dried avocado fruit powder

SP VirTis Benchtop K Freeze drier

Continuous Band Sealer

RESULTS

4 RESULTS

The results of the present study on "Post harvest characterisation and management of avocado (*Persea americana* Mill.)" accomplished in the Department of Post Harvest Technology, College of Agriculture, Vellanikkara during 2018-2021 are documented in this chapter under the following sections.

4.1. Characterisation of avocado accessions

4.2. Effect of shrink packaging and storage temperature on quality and shelf life of avocado.

4.3. Effect of food additives on quality of frozen avocado during storage

4.4. Effect of food additives on quality of avocado pulp and avocado fruit powder

4.1. Characterisation of avocado accessions

The unique pattern of flowering in 'A' type and 'B' type trees in avocado promotes cross pollination due to 'Protogynous Diurnally Synchronous Dichogamy (PDSD)' and the propagation through seeds have resulted in considerable genetic variability among the genotypes found in Kerala. Avocado fruits of each genotypes have defined and widely varying morphological and physico-chemical properties and nutritional contents. Even though more than 500 varieties of avocado have been reported in the world, most of them are not acceptable for commercial purposes due to different productivity problems, poor quality and susceptibility to post harvest handling (Dorantes *et al*., 2004). Therefore, selection of superior nutritional and morphological fruit characters will have immense significance in the commercial cultivation as well as in the consumer acceptability.

4.1.1 Horticultural traits

The results obtained for the morphological characterisation of fruits of twenty seven avocado accessions (Acc.) collected from Wayanad (Table 1), Idukki and Thrissur (Table 2) districts are presented in Table 1 and 2. Accessions 1 to 14 were collected from Wayanad (Plate 8.) and accessions 15 to 26 from Idukki district and accession 27 from Thrissur (Plate 9.). Each trait was evaluated in five fruits selected after sorting, according to 'Descriptors for Avocado' given by IPGRI (1995). Fruit shape varied from spheroid to clavate among the fruits of different genotypes.

Four accessions from Wayanad and one from Idukki had clavate shape, four accessions from Wayanad, three from Idukki and accession from Thrissur had narrowly

obovate shape. Five accessions had spheroid shape and four accessions had highly spheroid fruit shapes. While Acc. 14 and Acc. 23 had obovate, Acc. 9 had pyriform shape and Acc.15 and 24 had ellipsoidal shape.

Among the 27 accessions, lowest and highest fruit length (5.5 cm and 12.86 cm), fruit diameter (4.62 cm and 9.87 cm) and fruit weight (58.34 g and 640.41 g) were observed in accession 13 and 14, respectively. Thus significant variation was observed with respect to fruit size among the genotypes and maintained high uniformity in each accessions. Majority of the genotypes had inflated base; pointed and flattened base shapes were also observed while depressed fruit base was rare. Fruit apex was mostly rounded while flattened and slightly depressed were also seen. Both central and asymmetric apex positions were observed in different accessions. Pedicel position was commonly seen central on fruit. Ridges on fruit were absent in most of the genotypes, however fruits of accession 4 and 10 had entire ridges and accessions 8, 16 and 17 had partial ridges on fruit. Considering the gloss on fruit, most of the genotypes had strong glossiness on the fruit surface. Fruit skin surface was smooth in many of the genotypes while intermediate and rough fruit skin surface were also common. Purplish fruit skin colour were observed in most of the accessions collected from Wayanad while yellowish green skin colour was generally seen in accessions collected from Idukki and fruit skin thickness was observed in the range of 1-3 mm. Fruits of accessions from Idukki had thicker skin than that from Wayanad. Regarding the pliability of fruit skin, majority of the genotypes had brittle fruit skin. Adherence of skin to flesh was slight in many of the genotypes which favoured easy removal of fruit peel from the flesh. Intermediate and strong adherence were also observed. Colour of flesh next to skin was generally observed as greenish yellow while colour of flesh next to seed was light yellowish in the ripe fruits of majority of genotypes of avocado. Flesh texture of ripe fruits of most of the genotypes were buttery while there were a few fruits with pastose flesh. Avocado fruit flesh was low fibrous with neither sweet nor bitter in taste in most of the genotypes, but highly fibrous flesh was seen in Acc.22. General taste of flesh was found varying between excellent to poor and accessions 16, 19 and 25 had excellent taste. Degree of discolouration of cut opened fruit after 4 hours was observed only in accessions 3, 5, 14, 22 and 26 and the discolouration was brown for all accessions except accession 5 which showed grey discolouration. Variability in morphological characteristics could be summed up as the cumulative effect of genotype, environment and cultural practices.

Plate 8. Avocado accessions collected from Wayanad

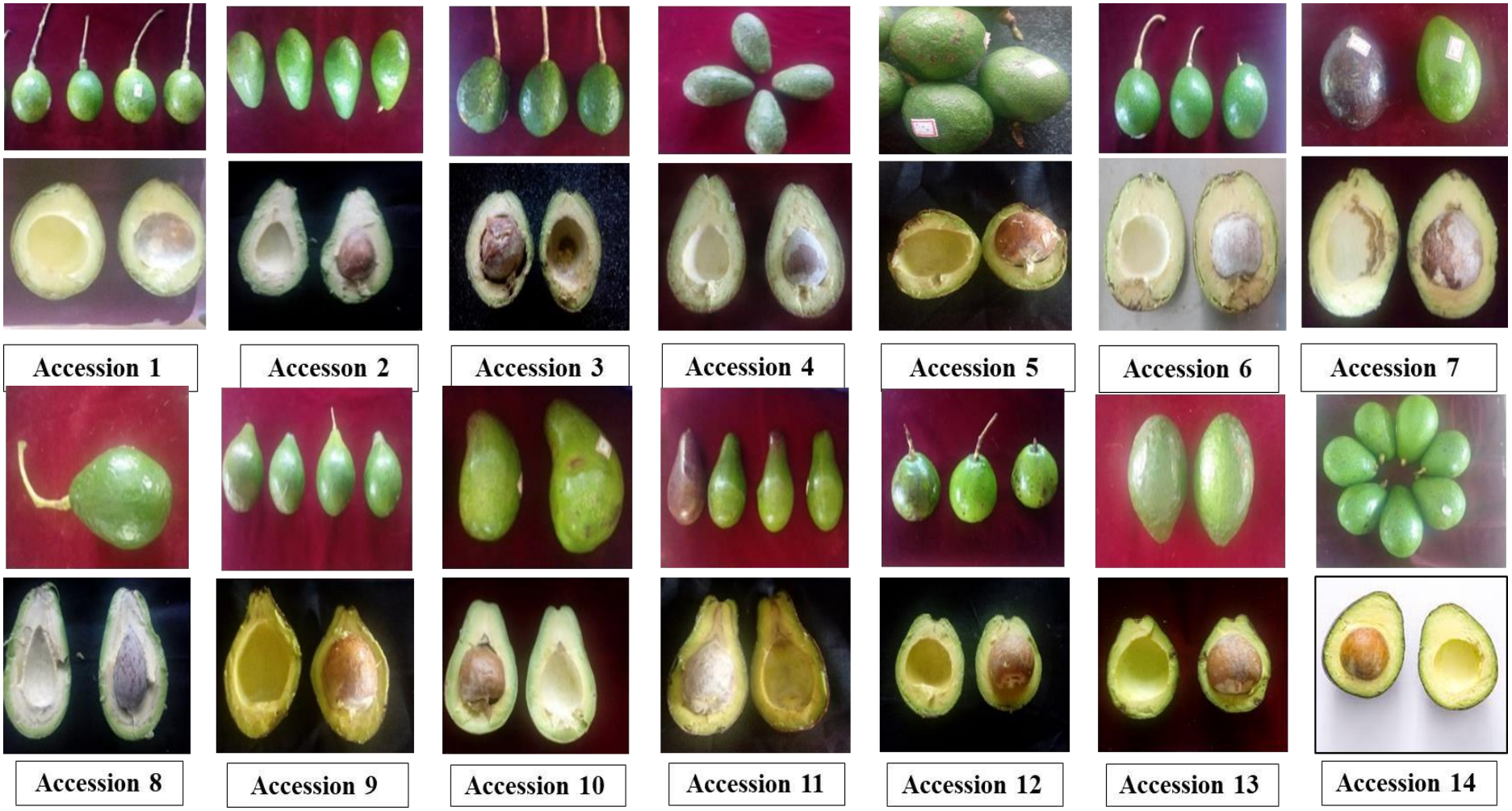


Plate 9. Avocado accessions collected from Idukki and Thrissur

Table 1. Characterisation based on horticultural traits of accessions in Wayanad

	Acc 1	**Acc 2**	**Acc 3**	**Acc 4**	**Acc 5**	**Acc 6**	**Acc 7**	**Acc 8**	**Acc 9**	**Acc 10**	**Acc 11**	**Acc 12**	**Acc 13**	**Acc 14**
Fruit shape	High spheroid	Clavate	Clavate	Clavate	Spheroid	Spheroid	Narrowly obovate	Narrowly obovate	Pyriform	Narrowly oboate	Clavate	Spheroid	Narrowly obovate	Obovate
Fruit length (cm)	6.08	11.18	12.76	8.62	6.68	6.86	8.60	8.52	11.04	6.92	6.73	6.58	5.50	12.86
Fruit diameter (cm)	5.61	6.37	7.00	5.15	6.59	7.08	6.94	6.74	6.71	6.15	7.00	6.68	4.62	9.87
Fruit weight (g)	89.42	247.80	312.38	113.78	163.70	182.50	209.06	209.16	224.92	329.98	312.42	149.68	58.34	640.41
Fruit size uniformity	High	Intermediate	High	High	High	High	High	High	Intermediate	High	Intermediate	High	Intermediate	High
Fruit base shape	Flattened	Inflated	Inflated	Inflated	Inflated	Flattened	Pointed	Pointed	Inflated	Inflated	Inflated	Inflate	Pointed	Flatte ned
Fruit apex shape	Flattened	Rounded	Rounded	Rounded	Rounded	Flattened	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Flatte ned
Fruit apex position	Asymmetric	Central	Central	Central	Asymmetric	Central	Central	Central	Asymmetric	Asymmetric	Central	Asymmetric	Central	Asymmetric
Ridges on fruit	None	None	None	Entire	None	None	None	Partial	None	Entire	None	None	None	None
Gloss on fruit	Medium	Strong	Strong	Weak	Weak	Strong	Strong	Medium	Weak	Strong	Strong	Strong	Weak	Medium
Pedicel position on fruit	Central	Asymmetric	Central	Asymmetric	Central	Central	Central	Central	Central	Central	Central	Central	Central	Asymmetric

Contd…

Fruit skin surface	Intermediate	Smooth	Smooth	Rough	Intermediate	Intermediate	Intermediate	Rough	Intermediate	Rough	Smooth	Smooth	Intermediate	Intermediate
Fruit skin colour	142 Strong yellowish purple	67A Strong purplish red	74A Deep reddish purple	142A Strong yellowish purple	142B Brillant yellowish green	142A Strong yellowish purple	77A Deep reddish purple	202A Dark grayish purple	79A Dark purple	141C Strong yellowish green	79C Deep purplish	64C Strong purplish red	80A Speckled Green Purple	66B Vivid purplish red
Fruit skin thickness (mm)	1.34	1.04	3.04	1.34	1.02	1.04	1.12	2.23	2.02	3.12	1.06	1.21	2.24	2.31
Pliability of fruit skin	Brittle	Brittle	Brittle	Pliable	Brittle	Pliable	Brittle	Brittle	Brittle	Brittle	Pliable	Pliable	Brittle	Brittle
Adherence of skin to flesh	Intermediate	Intermediate	Strong	Slight	Slight	Slight	Slight	Intermediate	Slight	Slight	Slight	Strong	Strong	Intermediate
Colour of flesh next to skin	141D Strong yellowish green	142C Light yellowish green	142A strong yellowish green	142D Light yellowish green	142B Brillant yellowish green	149A Brillant yellowish green	142A Strong yellowish green	149C Brillant yellowish green	142A Strong yellowish green	142B Brillant yellowish green	142A Strong yellowish Green	142A Strong yellowish Green	142A Strong yellowish Green	142A Strong yellowish green
Colour of flesh next to seed	1D Pale greenish yellow	154D Light yellowish green	2C light yellowish green	150D Light yellowish green	154D Light yellowish green	154B brilliant yellowish green	4C Light greenish yellow	2C Light yellowish green	2B Brillant greenish yellow	2C Light yellowish green	2C Light yellowish green	2C light yellowish green	2C light yellowish green	3D Light greenish yellow
Flesh texture	Buttery	Buttery	Buttery	Buttery	Pastose	Buttery	Buttery	Buttery	Pastose	Buttery	Buttery	Buttery	Buttery	Pastose

Contd…

Sweetness of flesh	Low	Intermediate	Intermediate	Low	Low	Low	Intermediate	Low	Low	Low	Intermediate	Low	Intermediate	Low
Bitterness of flesh	Low	Intermediate	Low	Low	Low	Low	Low	Low	High	High	Low	Intermediate	Low	Low
Fibre in flesh	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
General taste of flesh	Fair	Good	Good	Good	Fair	Fair	Fair	Fair	Poor	Poor	Good	Fair	Good	Fair
Degree of colouration of open fruit after 4hr.	Nil	Nil	High	Nil	High	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	High
Colour of discoloration	Nil	Nil	Brown	Nil	Grey	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Brown

Table 2. Characterisation based on horticultural traits of accessions in Idukki and Thrissur

	Acc 15	**Acc 16**	**Acc 17**	**Acc 18**	**Acc 19**	**Acc 20**	**Acc 21**	**Acc 22**	**Acc 23**	**Acc 24**	**Acc 25**	**Acc 26**	**Acc 27**
Fruit shape	Ellipsoid	Clavate	High spheroid	High spheroid	Narrowly obovalte	Narrowly obovalte	High spheroid	Spheroid	Obovate	Ellipsoid	Narrowly obovate	Spheroid	Narrowly obovate
Fruit length (cm)	7.75	10.50	8.95	8.70	9.65	8.85	9.30	8.60	11.10	9.20	9.50	7.28	12.06
Fruit diameter (cm)	6.82	6.87	8.02	7.98	7.20	7.29	8.97	8.02	8.02	7.61	7.69	7.81	7.14
Fruit weight (g)	188.92	247.74	305.36	310.84	267.97	247.27	395.00	275.39	335.00	273.66	480.10	242.94	452.40
Fruit size uniformity	Intermediate	High	Low	High	Interm ediate	Intermediate	High	High	High	High	High	High	High
Fruit base shape	Inflated	Pointed	Flattened	Flattened	Pointed	Pointed	Flattened	Depressed	Inflated	Inflated	Inflated	Depressed	Inflated
Fruit apex shape	Rounded	Rounded	Rounded	Flattened	Rounded	Slightly depressed	Flattened	Flattened	Round	Round	Round	Flattened	Round
Fruit apex position	Central	Central	Central	Asymmetric	Central	Asymmetric	Asymmetric	Central	Asymmetric	Central	Central	Central	Asymmetric
Ridges on fruit	Absent	Partial	Partial	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Contd…

Gloss on fruit	Medium	Weak	Medium	Medium	Medium	Strong	Weak	Strong	Strong	Medium	Weak	Strong	Medium
Pedicel position on fruit	Central	Central	Central	Central	Central	Central	Central	Asymmetric	Asymmetric	Central	Central	Central	Central
Fruit skin surface	Smooth	Rough	Intermediate	Rough	Rough	Smooth	Intermediate	Smooth	Smooth	Smooth	Intermediate	Smooth	Smooth
Fruit skin colour	202A Dark greyish purple	142A Strong yellowish green	142B Brillant yellowish green	142B Brillant yellowish green	154A Vivid yellowish green	202A Dark greyish purple	153C Strong greenish yellow	142A Strong yellowish green	142A Strong yellowish green	142A Strong yellowish green	186A Moderate purplish red	58C Strong purplish red	149A Brilliant yellowish green
Fruit skin thickness (mm)	3.08	2.03	2.52	3.03	3.04	3.05	3.06	1.04	2.03	3.06	2.55	2.06	1.02
Pliability of fruit skin	Brittle	Brittle	Brittle	Brittle	Brittle	Brittle	Brittle	Pliable	Brittle	Brittle	Brittle	Brittle	Pliable
Adherence of skin to flesh	Intermediate	Slight	Strong	Strong	Slight	Strong	Intermediate	Slight	Slight	Intermediate	Intermediate	Slight	Slight
Colour of flesh next to skin	142A Strong yellowish green	149A Bright yellowish green	142D Light yellowish green	142D Light yellowish green	144D Light yellowish green	154D Light yellowish green	142B Brillant yellowish green	142C Light yellowish green	142D Light yellowish green	142B Brillant yellowish green	142C Light yellowish green	142D Light yellowish green	149C Brilliant yellowish green

Contd…

Colour of flesh next to seed	3D Light greenish yellow	4D Pale yellowish	4D Pale yellowish	4D Pale yellowish	4D Pale yellowish	154D Light yellowish green	3D Light greenish yellow	4D Pale yellowish green	3D Light greenish yellow	3D Light greenish yellow	2C Light yellowish green	4D Pale yellowish green	1C Light greenish yellow
Flesh texture	Pastose	Pastose	Buttery	Buttery	Buttery	Buttery	Buttery	Pastose	Buttery	Buttery	Buttery	Buttery	Pastose
Sweetness of flesh	Intermediate	High	Intermediate	Intermediate	Intermediate	Low	Low	Intermediate	Intermediate	Low	Low	High	Low
Bitterness of flesh	Low	Low	Low	Low	Low	Low	High	Intermediate	Intermediate	Intermediate	Low	Low	Low
Fibre in flesh	Low	Low	Low	Intermediate	Low	Low	Low	High	Intermediate	Low	Low	Intermediate	Low
General taste of flesh	Good	Excellent	Good	Good	Excellent	Fair	Fair	Good	Good	Fair	Excellent	Fair	Fair
Degree of colouration of open fruit after 4hr.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	High	Nil	Nil	Nil	High	Nil
Colour of discolouration	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Brown	Nil	Nil	Nil	Brown	Nil

4.1.2 Biochemical parameters: The results obtained for the biochemical and nutritional constitution of fruits of avocado accessions are presented in Table 3.

4.1.2.1 Total soluble solids*:* Significant variation was noticed among genotypes with respect to their TSS content. Total soluble solids (TSS) were observed to be in the range of 4.27 ºBrix in Acc. 20 to 11.30 ºBrix in Acc. 13.

4.1.2.2 Titratable acidity: Titratable acidity varied significantly among the accessions which ranged from 0.28 to 2.84 % in Acc. 27 and Acc. 12, respectively.

4.1.2.3 Vitamin C: Significant difference in vitamin C content was observed among avocado genotypes. Vitamin C content in ripe avocado fruit varied from 5.33 mg/100g in Acc. 18 and Acc. 20, which was the lowest, to 20 mg/100g (highest) in Acc. 11.

4.1.2.4 Total carbohydrates: Total carbohydrates varied significantly among avocado genotypes. Total carbohydrates in ripe avocado fruits were comparatively low which were in the range of 0.40 g/100g in Acc. 7 (lowest) to 3.81 g/100g (highest) in Acc. 25.

4.1.2.5 Total protein: Total protein content showed significant variation among avocado genotypes and fruits collected from Wayanad (0.50 to 1.59 g/100g) had lower protein content than that from Idukki (8.33 to 11.93 g/100g). Total protein varied from 0.50 g/100g in Acc. 9 to 11.93 g/100g in Acc. 15.

4.1.2.6 Total phenols: Antioxidant potential of the plants are correlated with the content of phenolic compounds. Total phenols content were in the range of 33.33 mg/100g in Acc. 22 to 102.83 mg/100g in Acc. 12. It was comparatively higher in fruits collected from Wayanad.

4.1.2.7 Total flavonoid: Significant variation was not observed among genotypes with respect to their total flavonoid content. Total flavonoid content varied from 24.80 mg/100g in Acc. 9 to 66.67 mg/100g in Acc. 25.

4.1.2.8 Total fat: Fat content in avocado fruit is considered as the important maturity index which accumulated to maximum in optimum mature fruit. Total fat extracted from the fruits varied significantly from 0.79 to 10.02 % in Acc.4 and Acc.7, respectively.

4.1.2.9 Oleic acid*:* Oleic acid is the most predominant monounsaturated fatty acid with therapeutic value in avocado that ranged from 13.49 to 86.86 g/100g in Acc. 6 and Acc. 10, respectively which varied significantly among the genotypes.

4.1.2.10 Total ash*:* Ash content of the samples could be related with the mineral composition such as calcium, potassium, iron, *etc*. which have many health benefits.

Ash content of avocado fruits varied significantly from 0.27 % to 1.79 % in Acc. 8 and Acc. 3, respectively.

4.1.2.11 Calcium: Calcium content in fresh avocado fruits varied significantly among genotypes from 4.90 mg/100g in Acc. 16 to 13.46 mg/100g in Acc. 25.

4.1.2.12 Potassium*:* Potassium content in fresh avocado fruits varied significantly among genotypes. Avocado is a rich source of potassium than other fruits and it ranged from 122.27 mg/100g in Acc.16 to 460.00 mg/100g in Acc.10.

4.1.2.13 Iron*:* Iron content obtained in fresh and mature avocado fruits varied significantly which were in the range of 0.04 in Acc. 17 to 0.44 mg/100g in Acc. 26.

4.1.2.14 Crude fibre*:* Crude fibre content in fresh avocado fruits varied significantly among genotypes collected from different locations. Avocado fruits are rich in crude fibre content which was observed in the range of 2.24 to 7.61 % in Acc. 12 and Acc. 6, respectively.

Significantly higher TSS, Vitamin C, total phenols, total fat, oleic acid, potassium, total ash and crude fibre content were observed in accessions collected from Wayanad. Significantly higher total carbohydrates, total protein, total flavonoid, calcium and iron content were observed in accessions collected from Idukki. In some of the biochemical parameters, variations were observed among the accessions collected from Wayanad and Idukki districts. Comparatively high vitamin C, total phenols, potassium and ash content were observed in fruits collected from Wayanad and higher total protein, iron content as well as better horticultural traits were observed in accessions from Idukki. Comparing all the horticultural and biochemical parameters, highest total carbohydrates, total flavonoids and calcium content among the 27 accessions and highest vitamin C, potassium, total ash and crude fibre among accessions collected from Idukki were reported in accession 25 and it was selected as superior accession suitable for further post harvest management studies.

4.1.2.15 Antioxidant activity (DPPH, ABTS, FRAP)

Antioxidant activity of accession 25 was analysed in three different assays viz**.** 2, 2-Diphenyl 1-picrylhydrazyl (DPPH) radical assay, Ferric Reducing Antioxidant Potential (FRAP) assay and 2, 2-Azino-bis (3-ethylbenzthiazoline 6-sulfonic acid) (ABTS) assay (Table 4.). Radical scavenging activity was evaluated by IC_{50} (Inhibitory concentration). IC_{50} indicates the concentration of antioxidant in the sample capable for scavenging 50 % of the free radicals. Lower IC_{50} value indicates higher radical scavenging activity.

Table 3a. Biochemical characterisation of avocado accessions in Wayanad

Accessions	Total Soluble Solids (°Brix)	Titratable acidity (%)	Vitamin C (mg/100g)	Total carbohydrate (%)	Total protein (%)	Total phenols (mg/100g)	Total flavonoids (mg/100g)	Total fat (%)	Oleic acid (%)	Total ash (%)	Calcium (mg/100g)	Potassium (mg/100g)	Iron (mg/100g)	Crude fibre (%)
Acc 1	10.00	2.33	16.00	1.36	0.88	92.50	31.30	5.82	24.45	0.45	9.65	436.80	0.11	4.75
Acc 2	7.00	1.55	17.33	3.17	1.00	86.67	39.20	1.06	45.53	0.53	6.75	138.80	0.11	2.90
Acc 3	6.67	2.58	17.33	1.16	0.74	84.67	59.10	1.16	60.87	1.79	9.60	154.60	0.13	2.62
Acc 4	9.00	1.03	10.00	0.72	1.04	100.17	62.90	0.79	46.91	1.02	8.85	314.60	0.12	4.74
Acc 5	6.67	1.55	12.67	1.21	0.81	82.83	64.50	2.14	39.72	0.75	8.30	168.40	0.13	7.13
Acc 6	9.33	1.03	13.33	0.88	0.91	64.00	63.17	2.08	13.49	1.67	8.40	209.40	0.11	7.61
Acc 7	9.33	2.07	16.00	0.41	1.24	71.50	52.57	10.02	18.69	0.71	9.80	345.60	0.10	3.81
Acc 8	8.67	2.58	16.00	0.78	1.32	87.83	58.70	3.29	22.88	0.27	9.65	320.00	0.14	6.24
Acc 9	7.33	1.03	14.67	0.54	0.50	87.17	24.80	2.81	26.79	0.39	8.20	228.00	0.10	5.50
Acc 10	9.00	1.81	14.67	0.72	0.88	100.50	54.17	1.63	86.86	1.39	13.35	460.00	0.11	4.88
Acc 11	8.00	1.55	20.00	1.33	0.90	65.33	48.13	1.20	69.48	1.14	7.55	216.20	0.09	5.56
Acc 12	10.33	2.84	19.33	0.64	1.59	102.83	28.37	8.86	25.13	0.87	8.10	356.40	0.09	2.24
Acc 13	11.33	2.07	16.00	0.99	1.59	82.67	42.26	1.24	48.50	1.20	8.35	223.20	0.15	3.95
Acc 14	7.67	2.07	18.67	1.97	1.14	73.67	32.93	2.46	36.35	0.85	8.25	278.00	0.13	4.42

Contd… **Table 3b. Biochemical characterisation of avocado accessions in Idukki and Thrissur**

Accessions	Total Soluble Solids (°Brix)	Titratable acidity (%)	Vitamin C (mg/100g)	Total carbohydrate (%)	Total protein (%	Total phenols (mg/100g)	Total flavonoids (mg/100g)	Total fat (%)	Oleic acid (%)	Total ash (%)	Calcium (mg/100g)	Potassium (mg/100g)	Iron (mg/100g)	Crude fibre (%)
Acc 15	7.00	1.29	9.33	3.79	11.93	56.50	39.34	2.77	30.85	0.72	9.03	178.17	0.09	4.73
Acc 16	6.00	1.03	8.00	2.14	11.60	51.70	42.17	1.85	58.75	0.40	4.90	122.27	0.15	2.79
Acc 17	5.33	1.29	7.33	1.29	9.63	55.83	29.85	2.76	54.32	0.67	5.46	147.83	0.04	2.93
Acc 18	6.67	1.29	5.33	3.48	9.87	45.00	33.69	2.61	34.12	0.47	5.83	146.27	0.11	3.61
Acc 19	5.40	1.03	7.33	2.73	9.20	53.58	50.00	3.61	72.21	0.53	7.64	133.23	0.08	3.05
Acc 20	4.27	1.29	5.33	1.87	9.80	40.00	32.08	2.23	42.12	0.67	8.75	165.20	0.19	2.80
Acc 21	6.67	1.29	6.67	1.83	9.55	39.17	36.30	4.49	19.13	0.70	12.12	174.57	0.16	3.11
Acc 22	5.07	1.29	8.00	2.47	8.67	33.33	32.18	7.07	34.60	0.39	11.67	134.93	0.30	3.44
Acc 23	8.00	1.29	6.67	1.92	8.33	53.28	38.33	7.22	18.24	0.54	11.33	193.67	0.15	4.17
Acc 24	5.33	1.55	7.33	1.87	9.60	56.67	46.08	1.75	44.92	0.64	5.84	151.37	0.31	5.66
Acc 25	10.93	1.29	10.00	3.81	8.57	66.67	50.00	1.83	72.69	0.72	13.46	198.43	0.20	5.90
Acc 26	7.10	1.03	8.00	2.13	9.90	63.33	41.97	2.10	28.09	0.63	6.50	140.27	0.44	4.21
Acc 27	10.50	0.28	17.33	1.53	2.27	53.33	47.54	1.33	67.20	0.68	5.72	190.50	0.20	2.86
CD at 0.05%	**3.55**	**1.13**	**0.93**	**1.59**	**4.65**	**2.28**	**NS**	**1.29**	**0.94**	**0.52**	**3.42**	**1.22**	**0.16**	**0.93**

4.1.2.15.1. 2, 2-Diphenyl 1-picrylhydrazyl (DPPH) radical assay

DPPH radical scavenging activity of the methanolic extract of avocado fruit indicated the antioxidant potential due to water soluble compounds. In the DPPH assay, the IC_{50} value was reported as 4.07 μg/mL, which was higher than the IC_{50} value of standard ascorbic acid, 0.91 μg/mL.

4.1.2.15.2. Ferric Reducing Antioxidant Potential (FRAP) assay

In FRAP antioxidant assay, the potential antioxidant activity of a compound was indicated by its reducing capacity to convert ferric to ferrous (Fe^{3+} to Fe^{2+}) which was proportionate to the absorbance of the reaction mixture. In the methanolic extract of avocado fruit, radical scavenging activity was concentration dependent with IC_{50} value of 2.58 μg/mL which was higher than the IC_{50} value of standard ascorbic acid, 1.28 μg/mL.

4.1.2.15.3. 2, 2-Azino-bis (3-ethylbenzthiazoline 6-sulfonic acid) (ABTS) assay

In ABTS assay, $ABTS^{+}$ chromophore get decolourised by the oxidation of ABTS with ammonium persulphate by inhibiting the radical ABTS due to both water soluble and fat soluble antioxidant compounds in the sample. In the methanolic extract of avocado fruit, radical scavenging activity evaluated by ABTS assay indicated an IC_{50} value of 0.10 μg/mL. IC_{50} value of the sample was lower than standard ascorbic acid (1.02 μg/mL), indicating the higher antioxidant potential of the sample observed in ABTS assay.

Table 4. Antioxidant activity (IC_{50} value) of avocado fruit by DPPH, ABTS and FRAP assays

Antioxidant assay	Inhibitory concentration IC_{50} (μg/mL)	
	Sample (methanolic extract of the fruit)	Standard (Ascorbic acid)
DPPH assay	4.07	0.91
FRAP assay	2.58	1.28
ABTS assay	0.10	1.02

4.2. Effect of shrink packaging and storage temperature on quality and shelf life of avocado

Avocado fruits of nine treatments with calcium chloride pre-treatment and shrink packaging along with control stored under ambient condition (27-32 ºC), refrigeration (4-7 ºC) and cool chamber (12-13 ºC) are shown in Plate 10.

4.2.1. Physical and physiological parameters

4.2.1.1. Shelf life (days)

Avocado fruit was highly perishable with storage life of 7 days in control under ambient conditions without any packaging, while under refrigeration and cool chamber storage shelf life increased to 20 and 22 days, respectively. The result is shown in Table 5. In calcium chloride pre-treated fruits, shelf life of the fruits under ambient, refrigeration and cool chamber were 10, 20 and 24 days, respectively. It was observed that shrink packaging had important role in extending the shelf life of avocado as the shelf life was 15 days for calcium chloride pre-treated shrink packaged fruits stored under ambient condition. While pre-treatments with calcium chloride and shrink packaging along with storage under refrigeration (4-7 ºC) and cold chamber (12-13 ºC) were recorded with longest shelf life of 27 days.

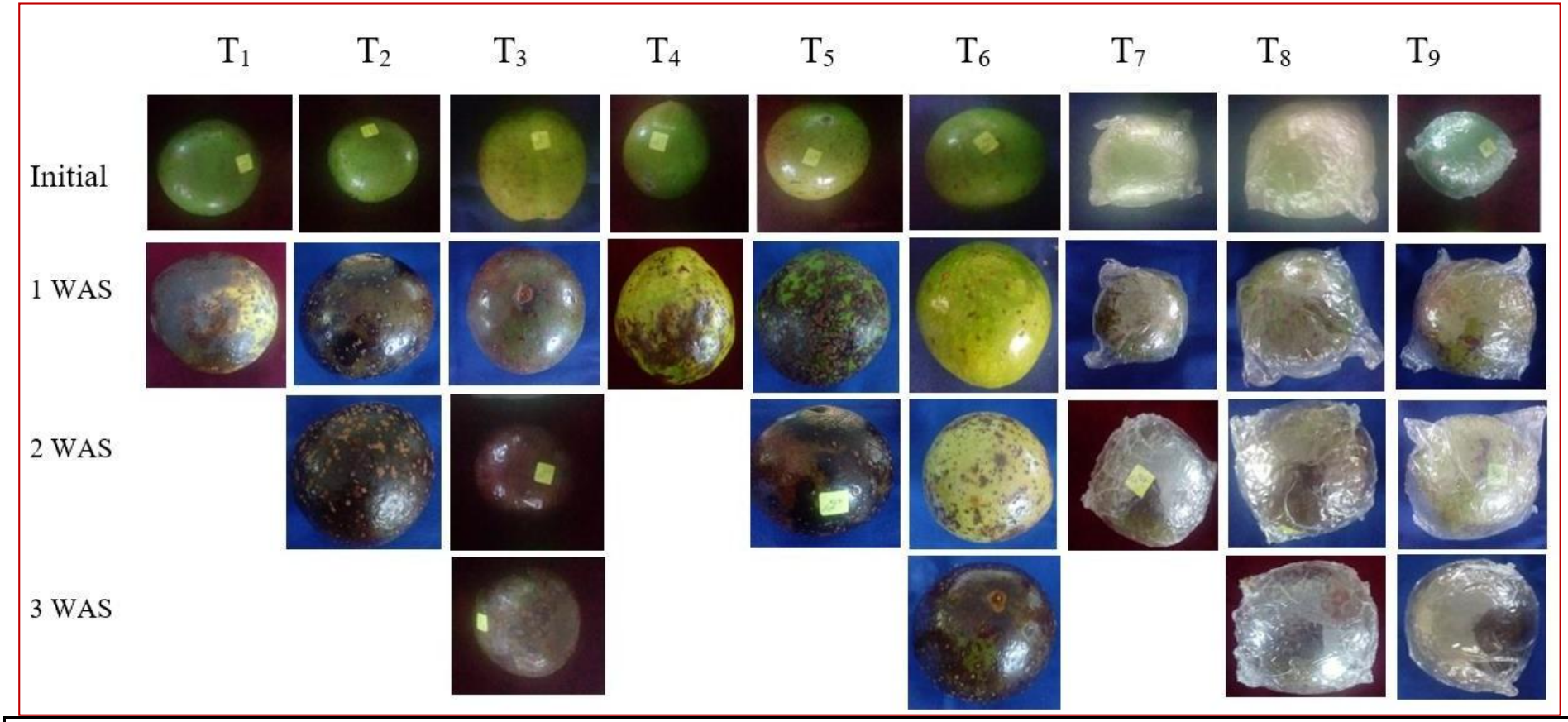
Table 5. Effect of shrink packaging and storage temperature on shelf life of avocado

Treatments	Shelf life (days)
T_1	7.00^{e}
T_2	20.00^{bc}
T_3	22.00^{ab}
T_4	10.00^{de}
T_5	20.00^{bc}
T_6	24.00^{ab}
T_7	15.00^{cd}
T_8	27.00^{a}
T_9	27.00^{a}
CD (0.05 %)	**6.34**

4.2.1.2. Physiological Loss in Weight (%)

It was observed that the Physiological Loss in Weight (PLW) increased during storage in all the treatments which were faster in control (T_1) without any treatment stored under ambient condition and it increased from 7.06 % to 12.95 % within 6 days after storage. Lower rate of PLW was noticed in shrink packaged fruits under refrigeration (T_8)

Plate 10. Avocado fruits during storage



T_1- Control + ambient condition
T_2- Control + refrigeration
T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition
T_5- $CaCl_2$ + refrigeration
T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink packaging + ambient condition
T_8- $CaCl_2$ + shrink packaging + refrigeration
T_9- $CaCl_2$ + shrink packaging + cool chamber
WAS: Weeks after storage

Table 6. Effect of shrink packaging and storage temperature on physiological loss in weight (PLW) (%) of avocado during storage

Treatments	3DAS	6DAS	9DAS	12DAS	15DAS	18DAS	21DAS	24DAS	27DAS
T_1	7.06^{ab}	12.95^{a}	*	*	*	*	*	*	*
T_2	2.07^{c}	4.26^{b}	6.95^{abc}	9.36^{ab}	10.83^{b}	10.75^{a}	*	*	*
T_3	1.58^{c}	3.06^{b}	4.90^{bc}	6.56^{b}	7.60^{b}	6.58^{ab}	7.54^{a}	*	*
T_4	7.40^{a}	12.93^{a}	18.17^{a}	*	*	*	*	*	*
T_5	2.36^{c}	4.91^{b}	8.82^{ab}	11.84^{a}	15.80^{a}	20.92^{a}	*	*	*
T_6	1.60^{c}	3.10^{b}	5.35^{bc}	7.05^{b}	8.19^{b}	7.94^{ab}	9.81^{a}	14.09^{a}	*
T_7	0.27^{c}	0.66^{c}	0.57^{c}	0.82^{c}	1.15^{bc}	*	*	*	*
T_8	0.05^{c}	0.07^{c}	0.43^{c}	0.65^{c}	0.45^{c}	0.47^{b}	0.48^{b}	0.79^{b}	0.89
T_9	0.30^{bc}	0.34^{c}	0.67^{c}	0.84^{c}	1.00^{bc}	0.76^{b}	0.77^{b}	1.08^{b}	1.28
CD (0.05 %)	**3.61**	**2.34**	**7.13**	**4.03**	**7.17**	**9.17**	**4.70**	**9.36**	

T_1- Control + ambient condition
T_2- Control + refrigeration
T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition
T_5- $CaCl_2$ + refrigeration
T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink packaging + ambient condition
T_8- $CaCl_2$ + shrink packaging + refrigeration
T_9- $CaCl_2$ + shrink packaging + cool chamber
DAS: Days after storage
* - Unmarketable
NS- Non-significant

Table 7. Effect of shrink packaging and storage temperature on respiration rate (% CO_2) of avocado during storage

Treatments	Initial	3DAS	6DAS	9DAS	12DAS	15DAS	18DAS	21DAS	24DAS	27DAS
T_1	1.49	1.43^{bc}	0.97^{b}	*	*	*	*	*	*	*
T_2		0.93^{bc}	0.43^{c}	0.40^{d}	0.40^{bc}	0.40	0.43^{d}	*	*	*
T_3		0.53^{c}	0.47^{c}	3.50^{b}	0.33^{c}	0.73	0.90^{bcd}	2.27	*	*
T_4		1.13^{bc}	0.80^{bc}	1.57^{cd}	*	*	*	*	*	*
T_5		2.37^{b}	0.60^{bc}	0.60^{d}	0.60^{b}	0.80	0.60^{cd}	*	*	*
T_6		0.70^{c}	0.70^{bc}	5.73^{a}	1.20^{a}	1.00	1.40^{ab}	1.93	1.37	*
T_7		7.73^{a}	5.43^{a}	2.27^{bc}	1.17^{a}	1.63	*	*	*	*
T_8		1.40^{bc}	0.40^{c}	0.43^{d}	0.30^{c}	0.37	1.20^{bc}	1.13	0.67	0.27
T_9		0.93^{bc}	0.63^{bc}	2.50^{bc}	1.10^{a}	2.53	2.00^{a}	1.77	1.50	1.37
CD (0.05 %)		**1.52**	**0.49**	**1.49**	**0.24**	**NS**	**0.73**	**NS**	**NS**	

T_1- Control + ambient condition T_2- Control + refrigeration T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition T_5- $CaCl_2$ + refrigeration T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink packaging + ambient condition T_8- $CaCl_2$ + shrink packaging + refrigeration T_9- $CaCl_2$ + shrink packaging + cool chamber
DAS: Days after storage * - Unmarketable NS- Non-significant

followed by cool chamber (T_9). In shrink packaged fruits under refrigeration (T_8), PLW increased from 0.05 to 0.89 % after 27 days of storage and in shrink packaged fruits under cool chamber (T_9), it was from 0.30 % to 1.28 % after 27 days of storage. The result is shown in Table 6.

4.2.1.3. Respiration rate (%)

Avocado fruits starts to ripen after harvest resulting in the rise of respiration rate and higher release of carbondioxide and ethylene, leading to fast degradation of fruits. Being a climacteric fruit, climacteric peak was observed in avocado fruits during storage in all the treatments (Table 7) which were more pronounced in control under cool chamber (T_3) (3.50 %), calcium chloride pre-treated fruits under cool chamber (T_6) (5.73 %) and calcium chloride treated shrink packaged fruits under cool chamber (T_9) (2.50 %) at 9 days after storage and in calcium chloride treated shrink packaged fruits under ambient storage (T_7) (7.73 %) at 3 days after storage. The least respiration rate with decreasing trend was noticed in fruits under refrigerated storage. Respiration rate of fruits kept as control under refrigeration (T_2) decreased from 0.93 to 0.43 % within 18 days of storage, in calcium chloride pre-treated fruits under refrigeration (T_5) from 2.37 to 0.60 % during 18 days of storage and in calcium chloride pre-treated shrink packaged fruits under refrigeration (T_8) from 1.40 to 0.27 % during 27 days of storage period. In fruits with longer shelf life such as shrink packaged fruits under cool chamber (T_9) and refrigeration (T_8), climacteric peak was observed towards the end of storage after 15 (2.53 %) and 18 (1.20 %) days of storage, respectively.

4.2.1.4. Ethylene evolution rate (%)

Ethylene evolution and respiration result in flesh softening and colour change of the fruit, indicating ripening of avocado fruits. In all the treatments, a peak in ethylene evolution rate was observed during storage which was more predominant in the early days of storage in control (Table 8). While fruits packaged in shrink packaging, ethylene evolution rate were low and the least ethylene evolution rate was seen in calcium chloride pre-treated fruits under cool chamber (T_6) decreased from 1.80 to 0.27 % during 24 days of storage. While in calcium chloride pre-treated shrink packaged fruits under refrigeration (T_8), a peak value was observed at 18 and 21 days after storage (3.23 %) and in calcium chloride pre-treated, shrink packaged fruits under cool chamber (T_9), after 24 days of storage (9.37 %). In fruits with longer shelf life such as shrink packaged fruits under refrigeration (T_8) and cool chamber (T_9), peak in ethylene

Table 8. Effect of shrink packaging and storage temperature on ethylene evolution rate (%) of avocado during storage

Treatments	Initial	3DAS	6DAS	9DAS	12DAS	15DAS	18DAS	21DAS	24DAS	27DAS
T_1	5.28	20.87^{b}	16.97^{b}	*						
T_2		6.33^{d}	9.80^{c}	12.23^{a}	12.03^{a}	15.67^{a}	28.30^{a}	*	*	*
T_3		12.53^{c}	6.70^{c}	12.30^{a}	10.33^{ab}	13.57^{b}	15.17^{b}	12.53^{a}	*	*
T_4		22.43^{a}	26.87^{a}	11.53^{a}				*	*	*
T_5		11.27^{c}	6.10^{cd}	6.00^{b}	7.90^{b}	6.87^{c}	8.30^{bc}	*	*	*
T_6		1.80^{e}	2.23^{de}	3.70^{bc}	0.40^{d}	0.53^{e}	1.07^{c}	0.27^{d}	0.27	*
T_7		1.33^{e}	6.27^{c}	9.60^{a}	3.23^{c}	3.00^{d}	*	*	*	*
T_8		2.27^{e}	2.50^{de}	2.13^{c}	2.03^{cd}	1.00^{de}	3.23^{c}	3.23^{b}	2.63	2.50
T_9		1.93^{e}	2.07^{e}	4.10^{bc}	1.57^{cd}	2.17^{de}	2.53^{c}	2.63^{c}	9.37	8.67
CD (0.05 %)		**1.42**	**3.91**	**3.58**	**2.77**	**2.08**	**7.54**	**0.57**	**0.43**	

T_1- Control + ambient condition T_2- Control + refrigeration T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition T_5- $CaCl_2$ + refrigeration T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink packaging + ambient condition T_8- $CaCl_2$ + shrink packaging + refrigeration T_9- $CaCl_2$ + shrink packaging + cool chamber
DAS: Days after storage * - Unmarketable NS- Non-significant

evolution was observed towards the end of storage, which indicated the slow rate of ripening.

4.2.1.5. Texture (kg/cm^2)

Texture is the resistance appeared when force is applied on the fruit indicating the quality and consumer acceptance of the fruit. Improper handling, unfavorable storage conditions adversely affect the texture and the decline in firmness corresponds to the end of storage life. The firmness of the fruits under storage decreased towards the end of storage.

Highest retention of firmness during storage was noticed in fruits under cool chamber and refrigeration. In cool chamber, texture of fruits kept as control (T_3) decreased from 11.01 to 0.29 kg/cm^2 during 21 days of storage, in calcium chloride pre-treated fruits (T_6) it decreased from 10.30 to 0.24 kg/cm^2 during 24 days of storage and in calcium chloride pre-treated shrink packaged fruits (T_9) from 6.60 to 0.33 kg/cm^2 during 27 days of storage. While in fruits under refrigeration, texture of the control (T_2) decreased from 9.34 to 1.79 kg/cm^2, in calcium chloride pre-treated fruits (T_5) it decreased from 10.88 to 0.12 kg/cm^2 during 18 days of storage and in calcium chloride pre-treated shrink packaged fruits (T_8) from 10.55 to 0.15 kg/cm^2 during 27 days of storage. The results are given in Table 9.

4.2.1.6. Decay (%)

Avocado fruits were noticed with physiological disorders such as grey pulp, pulp spot, mesocarp discoloration, in addition to the chilling injury and microbial attack, which lead to the decay of fruit during storage. It was noticed that the fruits stored as control without any packaging under ambient storage (T_1) decayed completely within 9 days after storage. Control (T_2) and calcium chloride pre-treated (T_5) fruits under refrigeration were reported with 100 per cent decay after 21 days and in those under cool chamber, 100 per cent decay were observed after 24 days of storage. Calcium chloride pre-treated shrink packaged fruits under refrigeration (T_8) and cool chamber (T_9) remained without 100 percent decay up to 27 days of storage, indicated the slow pace of decay of fruits (Table 10).

4.2.1.7. Post harvest disease incidence

One of the major loss of avocado is the result of post harvest disease which may have initiated from the field. During post harvest storage of avocado, with the end of

Table 9. Effect of shrink packaging and storage temperature on texture (kg/cm²) of avocado during storage

Treatments	Initial	3DAS	6DAS	9DAS	12DAS	15DAS	18DAS	21DAS	24DAS	27DAS
T_1	11.37	4.23^{d}	1.43^{d}	*	*	*	*	*	*	*
T_2		9.34^{b}	1.47^{d}	0.85	0.41^{e}	2.95	1.79^{ab}	*	*	*
T_3		11.01^{a}	4.64^{b}	3.89	3.18^{ab}	2.43	2.00^{ab}	0.29^{c}	*	*
T_4		1.16^{d}	0.55^{d}	4.46	*	*	*	*	*	*
T_5		10.88^{a}	3.20^{c}	0.30	4.16^{a}	0.27	0.12^{c}	*	*	*
T_6		10.30^{a}	4.20^{bc}	3.02	1.62^{d}	1.33	0.56^{bc}	0.31^{c}	0.24	*
T_7		1.03^{d}	0.64^{d}	0.67	2.90^{bc}	1.58	*	*	*	*
T_8		10.55^{a}	4.96^{b}	4.17	3.14^{ab}	1.81	1.21^{abc}	0.62^{a}	0.57	0.15
T_9		6.60^{c}	7.73^{a}	3.21	1.95^{cd}	2.50	2.58^{a}	0.47^{b}	0.38	0.33
CD (0.05 %)		**0.75**	**1.38**	**NS**	**1.08**	**NS**	**1.48**	**1.00**	**NS**	

T_1- Control + ambient condition T_2- Control + refrigeration T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition T_5- $CaCl_2$ + refrigeration T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink packaging + ambient condition T_8- $CaCl_2$ + shrink packaging + refrigeration T_9- $CaCl_2$ + shrink packaging + cool chamber
DAS: Days after storage * - Unmarketable NS- Non-significant

Table 10. Effect of shrink packaging and storage temperature on decay (%) of avocado during storage

	3 DAS	6DAS	9DAS	12DAS	15DAS	18DAS	21DAS	24DAS	27DAS
T_1	33.33	66.67	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00	100.00
T_2	0.00	0.00	33.33[c]	49.99[c]	66.66[b]	83.32[b]	100.00[a]	100.00	100.00
T_3	0.00	0.00	0.00	0.00	33.33[c]	49.99[ef]	66.66[c]	100.00	100.00
T_4	33.33	49.99	66.66[b]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00	100.00
T_5	0.00	0.00	0.00	0.00	33.33[c]	66.67[d]	100.00a	100.00	100.00
T_6	0.00	0.00	33.33[c]	46.66[c]	59.99[b]	73.32[c]	86.65[b]	100.00	100.00
T_7	0.00	0.00	33.33[c]	66.67[b]	100.00[a]	100.00[a]	100.00[a]	100.00	100.00
T_8	0.00	0.00	0.00	0.00	33.33[c]	46.22[f]	52.33[d]	66.67	100.00
T_9	0.00	0.00	0.00	0.00	33.33[c]	50.00[e]	66.67[c]	82.33	100.00
CD (0.05 %)	**NS**	**NS**	**8.43**	**7.28**	**18.47**	**3.82**	**2.44**	**NS**	**NS**

T_1- Control + ambient condition T_2- Control + refrigeration T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition T_5- $CaCl_2$ + refrigeration T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink packaging + ambient condition T_8- $CaCl_2$ + shrink packaging + refrigeration T_9- $CaCl_2$ + shrink packaging + cool chamber
DAS: Days after storage * - Unmarketable NS- Non-significant

storage, fruits were infected with diseases like anthracnose, fruit rot and stem end rot (Plate 11.). Anthracnose was developed as small brown circular lesions which turned into large dark brown or black decay. Stem end rot appeared as dark brown rot at the point of insertion of peduncle with off flavour development and became watery. Initially, fruit rot symptoms were observed as small irregular brown discolouration on the skin surface and brown streaks in the flesh along the vascular bundles and later entire fruit decayed with watery, off flavour development. From pathological studies, it was noticed that the fruits were infected with *Collectotrichum gloeosporioides*. Irregular brown spots with rough texture found on fruit were reported as scab growth caused by *Sphaceloma perseae* which affected the fruit even before harvest. In avocado, the important post harvest diseases were anthracnose, fruit rot and stem-end rot caused by *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* respectively (Bowen *et al*., 2018).

4.2.2. Biochemical parameters

4.2.2.1. Total Soluble Solids

Significantly the highest value of total soluble solids was observed in calcium chloride pre-treated fruits under cool chamber (T_6) throughout the storage period of 3 weeks, which increased from the initial value of 6.33 ºBrix to 9.13, 9.50 and 9.60 ºBrix at 1, 2 and 3 weeks after storage respectively. Wide variations were not seen in total soluble solids in avocado fruits during 3 weeks of storage among different treatments (Table 11a). Increase in TSS during storage indicated the ripening of fruits with the conversion of starch into soluble sugars. While lowest TSS content in calcium chloride pre-treated shrink packaged fruits under refrigeration (T_8) (5.03, 3.60 and 7.53 ^{0}Brix) and cool chamber (T_9) (6.13, 4.23 and 4.00 ^{0}Brix) at 1, 2 and 3 weeks after storage indicated the slow rate of ripening of fruits.

4.2.2.2. Titratable acidity

Titratable acidity decreased and remained non-significant among the treatments after one week of storage (Table 11a). Significantly lower titratable acidity (0.39 %) was recorded in control under refrigeration (T_2) after two weeks of storage. Higher titratable acidity observed in shrink packaged fruits indicated the lower rate of ripening. It increased towards the end of storage after three weeks in which calcium chloride pre-treated fruits under cool chamber (T_6) recorded significantly highest value (2.20 %).

Plate 11. Post harvest disease incidence in avocado

Anthracnose

Fruit rot

Stem end rot

Collectotrichum gloeosporoides

4.2.2.3. Total carbohydrates

The initial content of total carbohydrates at the time of storage was 3.29 g/100g which reduced during storage (Table 11a). Fruits stored as control under ambient condition (T_1) had highest total carbohydrates (2.66 g/100g) one week after storage. Calcium chloride per-treated fruits under refrigeration (T_5) (2.20 g/100g) and calcium chloride pre-treated, shrink packaged fruits under refrigeration (T_8) (0.82 g/100g) were recorded with higher content of total carbohydrates after 2 and 3 weeks of storage, respectively. Lower total carbohydrates were observed in calcium chloride pre-treated, shrink packaged fruits under cool chamber (T_9) (1.43 g/100g), calcium chloride pre-treated fruits under cool chamber (T_6) (0.73 g/100g) and T_9 (0.41 g/100g) after one, two and three weeks after storage, respectively.

4.2.2.4. Total protein

Total protein content in the avocado fruits increased after one week of storage and decreased towards the end of storage (Table 11a). Avocado fruits had an initial total protein content of 1.75 g/100g and after 1 and 2 weeks of storage significantly higher value (2.92 g/100g) was observed in calcium chloride pre-treated, shrink packaged fruits under refrigeration (T_8). After 3 weeks of storage significantly higher value was recorded in calcium chloride pre-treated fruits under cool chamber (T_6) (1.45 g/100g). Significantly lower total protein was noticed in calcium chloride pre-treated, shrink packaged fruits under ambient storage (T_7) after one week of storage (1.64 g/100g) and those under cool chamber (T_9) after two (1.51 g/100g) and three (0.79 g/100g) weeks of storage.

4.2.2.5. Vitamin C

Ascorbic acid content of avocado fruits before storage was about 14.67 mg/100g and after 1 week of storage it varied among the treatments without any significant difference. Higher ascorbic acid content was recorded in fruits stored in cool chamber without any treatment (T_3) (14.00 mg/100g) after two weeks of storage and calcium chloride pre-treated fruits in cool chamber (T_6) (16.00 mg/100g) three weeks after storage. Significantly lower values were observed in control (T_2) (11.33 mg/100g) and chloride pre-treated, shrink packaged fruits under refrigeration (T_8) (14.00 mg/100g) after two and three weeks of storage. It was observed that the ascorbic acid content decreased initially and increased with the end of storage (Table 11b).

Table 11a. Effect of shrink packaging and storage temperature on biochemical parameters of avocado during storage

Treatments	TSS (ºBrix)				Titratable acidity (%)				Total carbohydrate (g/100g)				Total protein (g/100g)			
	Initial	1WAS	2WAS	3WAS	Initial	1WAS	2WAS	3WAS	Initial	1WAS	2WAS	3WAS	Initial	1WAS	2WAS	3WAS
T_1	6.33	6.67^{de}	*	*	5.17	0.78	*	*	3.29	2.66^{a}	*	*	1.75	1.64^{c}	*	*
T_2		8.00^{bc}	9.00^{a}	*		0.49	0.39^{c}	*		2.01^{abc}	1.21^{bc}	*		1.99^{bc}	1.90^{cd}	*
T_3		7.27^{cd}	8.00^{a}	8.47^{ab}		0.52	0.52^{bc}	1.68^{b}		1.50^{c}	1.10^{bc}	0.62^{bc}		2.29^{b}	2.20^{bc}	1.20^{b}
T_4		6.93^{de}	*	*		0.39	*	*		1.56^{c}	*	*		2.90^{a}	*	*
T_5		6.53^{de}	7.67^{a}	*		0.52	0.65^{ab}	*		2.32^{ab}	2.20^{a}	*		2.21^{b}	2.13^{bc}	*
T_6		9.13^{a}	9.50^{a}	9.60^{a}		0.52	0.65^{ab}	2.20^{a}		1.63^{bc}	0.73^{cd}	0.62^{bc}		2.32^{b}	2.29^{b}	1.45^{a}
T_7		8.63^{ab}	9.00^{a}	*		0.36	0.78^{a}	*		1.94^{abc}	0.79^{cd}	*		1.64^{c}	1.63^{de}	*
T_8		5.03^{f}	3.60^{b}	7.53^{b}		0.39	0.78^{a}	1.55^{b}		1.92^{bc}	1.92^{ab}	0.82^{a}		2.92^{a}	2.92^{a}	0.67^{d}
T_9		6.13^{e}	4.23^{b}	4.00^{c}		0.65	0.78^{a}	1.55^{b}		1.43^{c}	1.37^{abc}	0.41^{c}		1.67^{c}	1.51^{e}	0.79^{c}
CD (0.05)		**0.86**	**2.71**	**1.47**		**NS**	**0.25**	**0.36**		**2.22**	**2.63**	**0.74**		**0.42**	**0.40**	**0.24**

T_1- Control + ambient condition T_2- Control + refrigeration T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition T_5- $CaCl_2$ + refrigeration T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink packaging + ambient condition T_8- $CaCl_2$ + shrink packaging + refrigeration T_9- $CaCl_2$ + shrink packaging + cool chamber
WAS: Weeks after storage * - Unmarketable NS- Non-significant

4.2.2.6. Total phenols

Total phenolic compounds increased in avocado fruits during 3 weeks of storage period (Table 11b). Initial total phenol content was 36.50 mg/100g which increased to 65.00 mg/100g in calcium chloride pre-treated fruits under ambient condition (T_4) after 1 week of storage and further increased to 70.00 and 85.83 mg/100g after 2 and 3 weeks, respectively in calcium chloride pre-treated, shrink packaged fruits under refrigeration at 4-7 ºC (T_8).

4.2.2.7. Total fat

With an initial fat content of 1.90 %, total fat content increased after one week of storage in all the treatments except calcium chloride pre-treated, shrink packaged fruits under ambient storage (T_7) and in cool chamber (T_9), and decreased towards the end of storage. Throughout the storage significantly highest total fat content was observed in calcium chloride pre-treated fruits stored under cool chamber (T_6) and it recorded 6.83, 6.55 and 4.85 % after one, two and three weeks of storage, respectively (Table 11b.).

By comparing the physical, physiological and biochemical parameters, calcium chloride pre-treated fruits with shrink packaging and stored under refrigeration at 4-7 ºC (T_8) was observed as the ideal pre-treatment for storage of avocado fruit. It had longer shelf life of 27 days with least PLW, lower respiration rate, ethylene evolution rate and decay with better retention of firmness. Less titratable acidity, higher total carbohydrates, total protein, total phenols were also observed in the treatment (T_8).

4.3. Effect of food additives on quality of frozen avocado during storage

Frozen avocado fruit slices pre-treated with sucrose (20-40 %), ascorbic acid (0.5 %) in combination with one of the preservatives (potassium metabisulphite, sodium benzoate, potassium sorbate at 0.1 %), packed in LDPE pouches and stored at -18 ºC for three months are shown in Plate 12.

4.3.1. Total Soluble Solids (ºBrix)

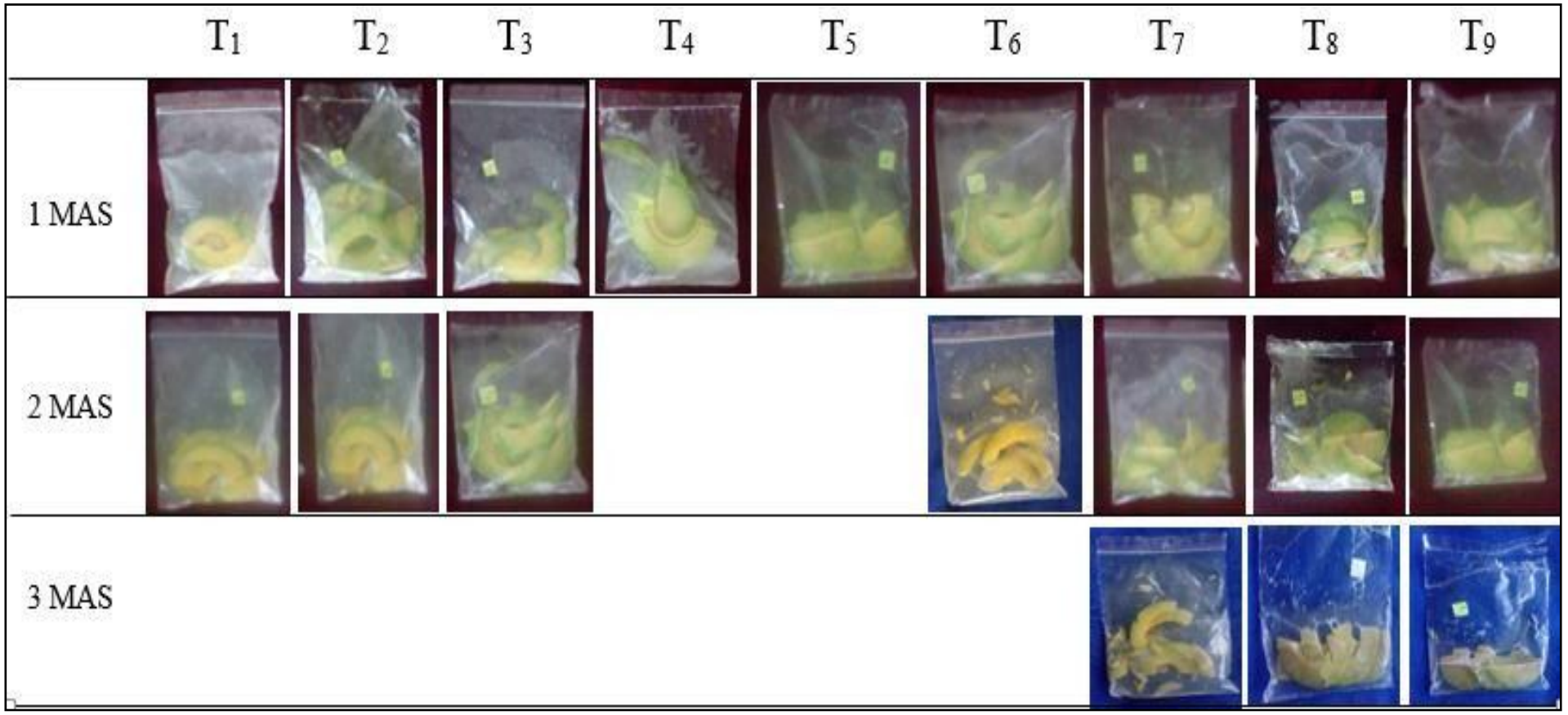
Initial TSS of avocado fruit was 6.33 ºBrix (Table 12a). Total soluble solids increased up to one month of storage and thereafter, decreased throughout the remaining storage period. Significantly higher total soluble solids were recorded in the fruit slices pre-treated with 40 % sucrose throughout the storage period. Fruit slices pre-treated with 40 % sucrose added with potassium metabisulphite (T_7) (26.00 ºBrix),

Table 11b. Effect of shrink packaging and storage temperature on biochemical parameters of avocado

Treatments	Vitamin C (mg/100g)				Total phenols (mg/100g)				Total fat (%)			
	Initial	1WAS	2WAS	3WAS	Initial	1WAS	2WAS	3WAS	Initial	1WAS	2WAS	3WAS
T_1	14.67	12.00	*	*	36.50	36.50^{cd}	*	*	1.90	2.63^{c}	*	*
T_2		12.00	11.33^{c}	*		45.50^{bcd}	57.00^{abc}	*		4.79^{b}	4.33^{b}	*
T_3		14.00	14.00^{a}	14.67^{ab}		29.50^{d}	51.33^{c}	76.60^{ab}		4.28^{b}	3.80^{b}	3.56^{ab}
T_4		13.33	*	*		65.00^{a}	*	*		5.21^{b}	*	*
T_5		10.00	12.00^{b}	*		35.00^{cd}	43.17^{c}	*		2.11^{c}	1.97^{c}	*
T_6		11.33	12.00^{b}	16.00^{a}		49.17^{abc}	55.83^{abc}	47.57^{c}		6.83^{a}	6.55^{a}	4.85^{a}
T_7		10.00	12.00^{b}	*		47.83^{abcd}	52.17^{bc}	*		1.76^{c}	1.40^{c}	*
T_8		10.67	12.00^{b}	14.00^{b}		56.50^{ab}	70.00^{a}	85.83^{a}		2.23^{c}	1.88^{c}	1.39^{c}
T_9		10.67	12.00^{b}	14.67^{ab}		51.83^{abc}	57.83^{abc}	58.50^{c}		1.49^{c}	1.55^{c}	1.19^{cd}
CD (0.05)		**NS**	**1.32**	**1.47**		**1.56**	**1.04**	**0.87**		**0.35**	**0.30**	**0.30**

T_1- Control + ambient condition T_2- Control + refrigeration T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition T_5- $CaCl_2$ + refrigeration T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink packaging + ambient condition T_8- $CaCl_2$ + shrink packaging + refrigeration T_9- $CaCl_2$ + shrink packaging + cool chamber
WAS: Weeks after storage * - Unmarketable NS- Non-significant

Plate 12. Pre-treated frozen avocado fruit slices during storage



T_1- Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%) T_2- Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
T_3- Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%) T_4- Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
T_5- Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%) T_6- Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
T_7- Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%) T_8- Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
T_9- Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%) MAS: Months after storage

potassium sorbate (T_9) (23.93 ºBrix) and sodium benzoate (T_8) (22.60 ºBrix) had the significantly higher TSS after one, two and three months of storage, respectively.

4.3.2. Titratable acidity (%)

Initial titratable acidity of the avocado fruit was 1.17 % (Table 12a.). Titratable acidity decreased during storage with proportionate increase in the concentration of sucrose. There was no significant difference in titratable acidity of avocado fruit slices among different treatments after one month of storage. Two months after storage, significantly higher titratable acidity (0.65 %) was noticed in avocado fruit slices pre-treated with 20 % sucrose and potassium metabisulphite (T_1) and lowest (0.26 %) in 40 % sucrose and potassium sorbate (T_9).

4.3.3. Total protein (g/100g)

Fresh avocado fruits had an initial total protein content of 0.87 g/100g (Table 12a.) and decreased during storage, followed by an increase towards the end of storage in fruit slices pre-treated with 40 % sucrose. Significantly higher total protein content (0.857, 0.782 and 1.43g/100g) was found in fruit slices pre-treated with 40 % sucrose and potassium metabisulphite (T_7) after 1, 2 and 3 months of storage, respectively. Significantly lower total protein content was observed in fruit slices pre-treated with 30 % sucrose and potassium metabisulphite (T_4) (0.40 g/100g) and 30 % sucrose and sodium benzoate (T_5) (0.43 g/100g) one week after storage. Two weeks after storage, fruit slices pre-treated with 20 % sucrose and potassium sorbate (T_3) and 30 % sucrose and potassium sorbate (T_6) had significantly lower (0.36 g/100g) total protein content.

4.3.4. Vitamin C (mg/100g)

Initial vitamin C content of avocado fruit was 14.67 mg/100g (Table 12a.). Vitamin C content increased in the initial phase of storage and decreased towards the end of storage. Significantly higher vitamin C content was recorded in samples pre-treated with 40 % sucrose and KMS (T_7) (92.00 mg/100g) after one month of storage. Avocado fruit slices pre-treated with potassium sorbate at different concentrations (20, 30 and 40 %) of sucrose had comparatively low vitamin C content (T_3, T_6 and T_9) after one month of storage. After 2 weeks of storage, significantly higher vitamin C (62.67 mg/100g) was noticed in fruit slices pre-treated with 40 % sucrose and sodium benzoate (T_8) and lower (16.00 mg/100g) in fruit slices pre-treated with 30 % sucrose and potassium sorbate (T_6).

Table 12a. Effect of food additives on quality of frozen avocado during storage

Treatments	TSS (^{0} Brix)				Titratable acidity (%)				Total protein (g/100g)				Vitamin C (mg/100g)			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_1	6.33	18.80^{cde}	17.33^{d}	*	1.17	0.78	0.65^{a}	*	0.87	0.76^{abc}	0.63^{b}	*	14.67	17.33^{e}	18.67^{b}	*
T_2		18.60^{de}	17.33^{d}	*		0.52	0.32^{bc}	*		0.70^{bc}	0.62^{b}	*		42.67^{c}	20.00^{b}	*
T_3		20.20^{cd}	17.00^{f}	*		0.78	0.27^{c}	*		0.70^{bc}	0.36^{c}	*		14.67^{e}	20.00^{b}	*
T_4		19.00^{cde}	*	*		0.78	*	*		0.40^{d}	*	*		62.67^{b}	*	*
T_5		17.00^{e}	*	*		0.78	*	*		0.43^{d}	*	*		34.67^{cd}	*	*
T_6		20.93^{bc}	13.93^{e}	*		0.52	0.52^{ab}	*		0.80^{ab}	0.36^{c}	*		18.67^{e}	16.00^{b}	*
T_7		26.00^{a}	20.20^{c}	17.73^{b}		0.52	0.52^{ab}	0.32		0.85^{a}	0.78^{a}	1.43		92.00^{a}	49.33^{a}	13.33
T_8		22.87^{b}	22.07^{b}	22.60^{a}		0.52	0.32^{bc}	0.25		0.72^{bc}	0.68^{ab}	1.21		81.33^{a}	62.67^{a}	16.00
T_9		25.67^{a}	23.93^{a}	16.27^{b}		0.52	0.26^{c}	0.19		0.63^{c}	0.63^{b}	1.16		25.33^{de}	20.00^{b}	16.00
CD (0.05)		**2.24**	**1.02**			**NS**	**0.25**			**3.29**	**2.15**			**1.27**	**0.59**	

T_1- Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%) T_2- Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
T_3- Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%) T_4- Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
T_5- Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%) T_6- Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
T_7- Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%) T_8- Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
T_9- Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%)
MAS: Months after storage * - Unmarketable NS- Non-significant

4.3.5. Total phenols (mg/100g)

Avocado fruit had an initial total phenols content of 36.50 mg/100g (Table 12b). Total phenols increased during storage, followed by a decrease towards the end of storage and significantly higher values were observed in fruit slices pre-treated with 30 % sucrose and KMS (T_4) (96.67 mg/100g) and 30 % sucrose and potassium sorbate (T_6) (98.30 mg/100g) after 1 and 2 months of storage, respectively. Lowest total phenolic content was observed in fruit slices pre-treated with 40 % sucrose and potassium metabisulphite (T_7) (56.67 mg/100g) after 1 month of storage. After 2 months of storage, fruit slices pre-treated with 20 % (T_3) and 40 % sucrose (T_9) with potassium sorbate, (76.67 mg/100g) had lowest total phenolic content.

4.3.6. Total carbohydrate (g/100g)

Avocado had an initial total carbohydrate content of 9.87 g/100g which decreased in frozen avocado slices during storage (Table 12b). Fruit slices pre-treated with 40 % sucrose and potassium metabisulphite (T_7) had significantly higher total carbohydrate (10.17 g/100g) and lower value (3.37 g/100g) was observed in fruit slices pre-treated with 30 % sucrose and potassium sorbate (T_6), after one month of storage. After two months of storage, total carbohydrate was significantly higher in samples pre-treated with 40 % sucrose added with potassium metabisulphite (T_7) (8.20 g/100g), sodium benzoate (T_8) (8.13 g/100g) and potassium sorbate (T_9) (8.43 g/100g).

4.3.7. Total fat (%)

Avocado had an initial fat content of 1.90 % and it increased after one month of storage in fruit slices pre-treated with 20 % sucrose and sodium benzoate (T_2), 30 % sucrose with potassium metabisulphite (T_4) and potassium sorbate (T_6) and 40 % sucrose with sodium benzoate (T_8) and potassium sorbate (T_9). After two and three months of storage, total fat content decreased in all the pre-treated fruit slices. Total fat content varied significantly among the treatments after 1 and 2 months of storage. Frozen fruit slices pre-treated with 20 % sucrose and potassium metabisulphite (T_1) had the lower (0.90 %) fat content and 30 % sucrose and potassium sorbate (T_6) had the higher (4.25 %) after one month of storage. After two months of storage, 30 % sucrose and potassium sorbate (T_6) (2.60 %) and fruit slices pre-treated with 40 % sucrose and sodium benzoate (T_8) (0.44 %) had significantly highest and lowest total fat content respectively (Table 12b.).

Table 12b. Effect of food additives on quality of frozen avocado during storage

Treatments	Total phenols (mg/100g)				Total carbohydrate (g/100g)				Total fat (%)			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_1	36.50	80.00^{abc}	95.00^{bc}	*	9.87	5.03^{de}	4.73^{c}	*	1.90	0.90^{d}	0.53^{d}	*
T_2		70.00^{bcd}	85.00^{c}	*		5.20^{cde}	7.30^{ab}	*		3.30^{ab}	1.34^{b}	*
T_3		70.00^{bcd}	76.67^{c}	*		5.90^{bcd}	5.50^{bc}	*		1.39^{cd}	1.06^{bc}	*
T_4		96.67^{a}	*	*		8.13^{ab}	*	*		2.11^{bc}	*	*
T_5		78.33^{abcd}	*	*		6.43^{bcd}	*	*		1.51^{cd}	*	*
T_6		85.33^{ab}	98.30^{a}	*		3.37^{e}	7.20^{ab}	*		4.25^{a}	2.60^{a}	*
T_7		56.67^{d}	85.00^{c}	55.00		10.17^{a}	8.20^{a}	5.53		1.32^{cd}	0.61^{cd}	0.36
T_8		76.67^{abcd}	96.70^{b}	48.33		5.50^{cde}	8.13^{a}	6.47		3.92^{a}	0.44^{de}	0.69
T_9		63.33^{cd}	76.67^{c}	48.33		7.43^{bc}	8.43^{a}	5.27		3.62^{a}	0.55^{d}	1.67
CD (0.05)		**1.19**	**1.22**			**2.25**	**1.89**			**1.20**	**0.49**	

T_1- Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%) T_2- Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
T_3- Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%) T_4- Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
T_5- Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%) T_6- Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
T_7- Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%) T_8- Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
T_9- Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%)
MAS: Months after storage * - Unmarketable NS- Non-significant

4.3.8. Peroxide value

Avocado fruit slices had an initial peroxide value of 12.64 meq O_2/kg (Table 12c.). After one month of storage, highest peroxide value (71.11 meq O_2/kg) was recorded in frozen fruit slices pre-treated with 30 % sucrose and potassium sorbate (T_6). The lowest peroxide value was observed in frozen fruit slices pre-treated with 40 % sucrose and KMS (T_7) after one (15.56 meq O_2/kg) and two (22.64 meq O_2/kg) months after storage. Significantly lower peroxide values were obtained in all the samples preserved with potassium metabisulphite. The oxidative deterioration of avocado slices was marginal in all the samples except the rancid taste towards the end of storage.

4.3.9. Polyphenol oxidase activity

Polyphenol oxidase (PPO) activity increased with increase in the period of storage and lower enzyme activity was observed in fruit slices pre-treated with 40 % sucrose and potassium sorbate (T_9) after one (20.00 unit/ml) and two (65.00 unit/ml) months of storage. Fruit slices pre-treated with 40 % sucrose and potassium metabisulphite (T_7) had lower polyphenoloxidase activity after three months of storage (127.00 unit/ml). All the fruit slices pre-treated with higher concentration sucrose had relatively lower enzymatic activity and subsequent browning during the storage (Table 12c.).

4.3.10. Water activity

Water activity of the fresh avocado fruit sample was 0.998 (Table 12c.). A decrease in water activity was noticed in the initial phase of storage and thereafter it increased till the end of storage. After one and two months of storage, significantly lower water activity (0.956 and 0.965) was noticed in fruit slices pre-treated with 40 % sucrose and potassium metabisulphite (T_7) and higher (0.995 and 0.999) in fruit slices pre-treated with 20 % sucrose and sodium benzoate (T_2), respectively. The effect of higher concentration of sucrose and potassium metabisulphite in the preservation of frozen avocado slices by lowering the water activity is evident from the result obtained.

4.3.11. Microbial population

Initial bacterial count observed in fresh avocado sample was $0.15x10^5$ cfu/g. Bacterial population was not detected in fruit slices pre-treated with 40 % sucrose added with potassium metabisulphite (T_7) and sodium benzoate (T_8) throughout the storage.

Table 12c. Effect of food additives on quality of frozen avocado during storage

Treatments	Water activity				Peroxide value (meq O_2/kg)				Polyphenol oxidase activity (unit/ml)			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_1	0.998	0.983^{b}	0.994^{ab}	*	12.64	17.12^{b}	88.37^{a}	*	15.00	252.50^{a}	500.00^{a}	*
T_2		0.995^{a}	0.999^{a}	*		21.24^{b}	88.28^{a}	*		162.50^{b}	407.50^{a}	*
T_3		0.978^{c}	0.998^{ab}	*		62.90^{a}	54.88^{ab}	*		132.50^{bc}	497.50^{a}	*
T_4		0.978^{c}	*	*		33.29^{b}	*	*		270.00^{a}	*	*
T_5		0.985^{b}	*	*		36.58^{b}	*	*		45.00^{d}	*	*
T_6		0.979^{c}	0.998^{ab}	*		71.11^{a}	88.00^{a}	*		37.50^{d}	340.00^{a}	*
T_7		0.956^{e}	0.965^{d}	0.982		15.56^{b}	22.64^{bc}	20.73		45.00^{d}	91.00^{b}	127.50
T_8		0.958^{e}	0.981^{c}	0.998		33.39^{b}	33.27^{bc}	29.32		75.00^{cd}	140.00^{b}	162.50
T_9		0.964^{d}	0.992^{bc}	0.993		17.18^{b}	36.44^{bc}	13.47		20.00^{d}	65.00^{b}	162.50
CD (0.05)		**0.004**	**0.012**			**2.02**	**2.18**			**66.53**	**176.09**	**NS**

T_1- Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%) T_2- Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
T_3- Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%) T_4- Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
T_5- Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%) T_6- Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
T_7- Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%) T_8- Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
T_9- Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%)
MAS: Months after storage * - Unmarketable NS- Non-significant

Table 13. Effect of food additives on microbial population of frozen avocado during storage

Treatments	Microbial population (bacteria, fungi and yeast)											
	Initial			1MAS			2MAS			3MAS		
	B	Y	F	B	Y	F	B	Y	F	B	Y	F
T_1	1.5	0	0	2.3^{b}	0.0	2.0^{a}	3.3^{bc}	1.0	13.7^{a}	*	*	*
T_2				0.0^{d}	0.0	0.0	2.0^{cd}	0.0	2.0^{b}	*	*	*
T_3				2.7^{ab}	0.0	1.0^{b}	4.3^{b}	0.0	1.0^{b}	*	*	*
T_4				2.3^{b}	0.0	1.0^{b}	*	*	*	*	*	*
T_5				4.0^{a}	0.0	0.0	*	*	*	*	*	*
T_6				2.0^{bc}	0.0	1.0^{b}	7.7^{a}	0.0	3.0^{b}	*	*	*
T_7				0.0^{d}	0.0	0.0	0.0	1.0	0.0	0	7.0	24.0
T_8				0.0^{d}	0.0	1.0^{b}	0.0	0.0	11.7^{a}	0	0.0	43.7
T_9				1.7^{bcd}	0.0	0.0	2.0^{cd}	0.0	0.0	13.3	0.0	1.3
CD (0.05)				**1.43**	**NS**	**0.73**	**1.79**	**NS**	**4.25**	**NS**	**NS**	**NS**

T_1- Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%) T_2- Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
T_3- Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%) T_4- Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
T_5- Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%) T_6- Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
T_7- Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%) T_8- Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
T_9- Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%)
MAS: Months after storage * - Unmarketable
B: Bacteria (10^5 cfu/g) Y: Yeast (10^4 cfu/g) F: Fungi (10^3 cfu/g)

Yeast colonies were not observed in any treatments after one month of storage. In treatments T_2 (fruit slices pre-treated with 20 % sucrose and sodium benzoate), T_5 (fruit slices pre-treated with 30 % sucrose and sodium benzoate), T_7 (fruit slices pre-treated with 40 % sucrose and potassium metabisulphite) and T_9 (fruit slices pre-treated with 40 % sucrose and potassium sorbate), fungal population was not detected, after one month of storage. After 2 months, no fungal count was seen in T_7 (fruit slices pre-treated with 40 % sucrose and potassium metabisulphite) and T_9 (fruit slices pre-treated with 40 % sucrose and potassium sorbate). Yeast population was absent in all the samples except T_1 (fruit slices pre-treated with 20 % sucrose and potassium metabisulphite) and T_7 (fruit slices pre-treated with 40 % sucrose and potassium metabisulphite) ($1x10^4$ cfu/10g) after two months of storage. After three months of storage, yeast population ($7x10^4$ cfu/10g) was observed only in T_7 (fruit slices pre-treated with 40 % sucrose and potassium metabisulphite).

Lower microbial population was noticed in fruit slices pre-treated with 40 % sucrose and potassium metabisulphite (T_7) throughout the storage. Both bacterial and yeast colonies were absent in fruit slices pre-treated with 40 % sucrose and sodium benzoate (T_8) throughout the storage. In fruit slices pre-treated with 40 % sucrose and potassium sorbate (T_9) yeast and fungal population remained low throughout the storage (Table 13).

4.3.12. Organoleptic evaluation

After one month storage of frozen avocado fruit slices, highest consumer acceptance with total scores of 64.1 was noticed in T_3 (fruit slices pre-treated with 20 % sucrose and potassium sorbate) followed by T_7 (fruit slices pre-treated with 40 % sucrose and KMS) (64) as shown in the Table 14. After two months of storage, T_7 obtained total scores 60.8 which was the highest and it reduced to 56.2 after 3 months. Fruit slices pre-treated with T_7 retained higher organoleptic scores throughout the storage. Even though the organoleptic qualities of the frozen avocado slices decreased towards the end of storage due to the rancidity, off-flavour and reduction in texture, fruit slices pre-treated with 40% sucrose remained acceptable up to 3 months of storage.

Considering the higher TSS (26.00 ^oBrix), vitamin C (92.00 mg/100g), total carbohydrates (10.17 g/100g), total protein (0.85 to 1.43 g/100g) and organoleptic acceptability along with lower values for total phenols (56.67 mg/100g), water activity

Table 14. Effect of food additives on organoleptic scores of frozen avocado during storage

Organoleptic evaluation																										
Treatments	1MAS									2MAS									3MAS							
	A	C	Te	F	O	Ta	At	Oa	Ts	A	C	Te	F	O	Ta	At	Oa	Ts	A	C	Te	F	O	Ta	At	Oa	Ts
T_1	7.8	8	8.6	7.2	8.5	7.8	6.6	8.5	63.0	7.3	7.4	7.0	6.7	6.1	6.9	6.1	7.1	54.6	*	*	*	*	*	*	*	*	*
T_2	7.2	7.1	7.4	7.4	7.7	7.2	7.4	7.2	58.6	5.4	4.7	6.5	6.2	5.8	6.3	5.6	6.0	46.5	*	*	*	*	*	*	*	*	*
T_3	8.3	8.1	7.3	8.4	8.5	7.5	7.9	8.1	64.1	7.5	7.5	7.2	7.0	7.1	7.2	6.8	7.4	57.7	*	*	*	*	*	*	*	*	*
T_4	7.3	7.5	6.7	7.4	6.9	7.5	6.1	7.5	56.9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
T_5	8	7.3	8.6	7.9	7.3	7.4	6.7	7.7	60.9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
T_6	7.5	7.4	8.3	8.1	7.1	7.6	7.6	7.7	61.3	5.3	4.9	6.0	5.8	6.1	7.2	5.6	6.1	47	*	*	*	*	*	*	*	*	*
T_7	7.9	8.2	8.0	8.2	8.0	7.9	7.6	8.2	64.0	7.9	8.2	7.3	7.3	7.1	8.8	6.6	7.6	60.8	7.4	7.6	6.8	7.4	6.2	6.0	6.6	7.2	56.2
T_8	7.9	7.3	7.4	7.8	8.4	7.5	7.9	7.9	62.1	6.9	6.7	6.4	6.0	6.4	6.2	5.5	6.7	50.8	6.0	5.8	6.2	6.8	5.4	6.0	5.4	5.8	48.4
T_9	7.6	8.1	7.9	7.7	7.4	7.3	7.5	7.9	61.4	6.7	6.7	6.7	6.5	6.6	6.6	5.7	6.8	52.3	6.2	6.2	6.4	7.0	6.0	6.0	5.8	6.0	53.6
Kendall's W Test	**0.117**	**0.177**	**0.216**	**0.15**	**0.27**	**0.125**	**0.31**	**0.108**		**0.748**	**0.791**	**0.273**	**0.194**	**0.139**	**0.104**	**0.218**	**0.446**		**0.41**	**0.547**	**0.285**	**0.04**	**0.216**	**0.261**	**0.037**	**0.203**	

T - Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%) T - Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
1 2

T - Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%) T - Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
3 4

T - Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%) T - Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
5 6

T - Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%) T - Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
7 8

T - Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%) MAS: Months after storage * - Unmarketable
9

A: Appearance C: Colour Te: Texture F: Flavour Ta: Taste At: After taste O: Odour Oa: Overall acceptability Ts: Total score

(0.956 to 0.982), peroxide value (15.56 to 22.64 meq./kg) and microbial population, frozen avocado slices added with 40 % sucrose, 0.5 % ascorbic acid and 0.1 % KMS (T_7) was observed as the ideal pre-treatment for the preservation of frozen avocado slices for three months.

4.3.13. Cost of production

The total cost for the preparation of one kilogram frozen avocado fruit slices pre-treated with 40 % sucrose, 0.5 % ascorbic acid and 0.1 % KMS and packed in 200 gauge LDPE pouches was about Rs. 450.23/- (Table 15)

Table 15. Cost of production of 1 kg frozen avocado slices

Frozen avocado slices	Rate	Quantity	Cost (Rs.)
Avocado fruits	Rs.200/kg	1.25 kg	250.00
Blast freezer (1kWh)	Rs.5/unit	30 min	2.50
Sucrose (20-40 %)	Rs.200/500 g	400 g/kg	150.00
Ascorbic acid (0.5 %)	Rs.300/500 g	5 g	3.00
KMS (0.1%)	Rs.368/500 g	1 g	0.73
LDPE pouches (200 gauge)	Rs.100/kg	4 no.	4.00
Labour charge	600/8hr	2 hr	40.00
Total cost			**Rs.450.23/kg**

4.4. Effect of food additives on quality of avocado pulp and avocado fruit powder

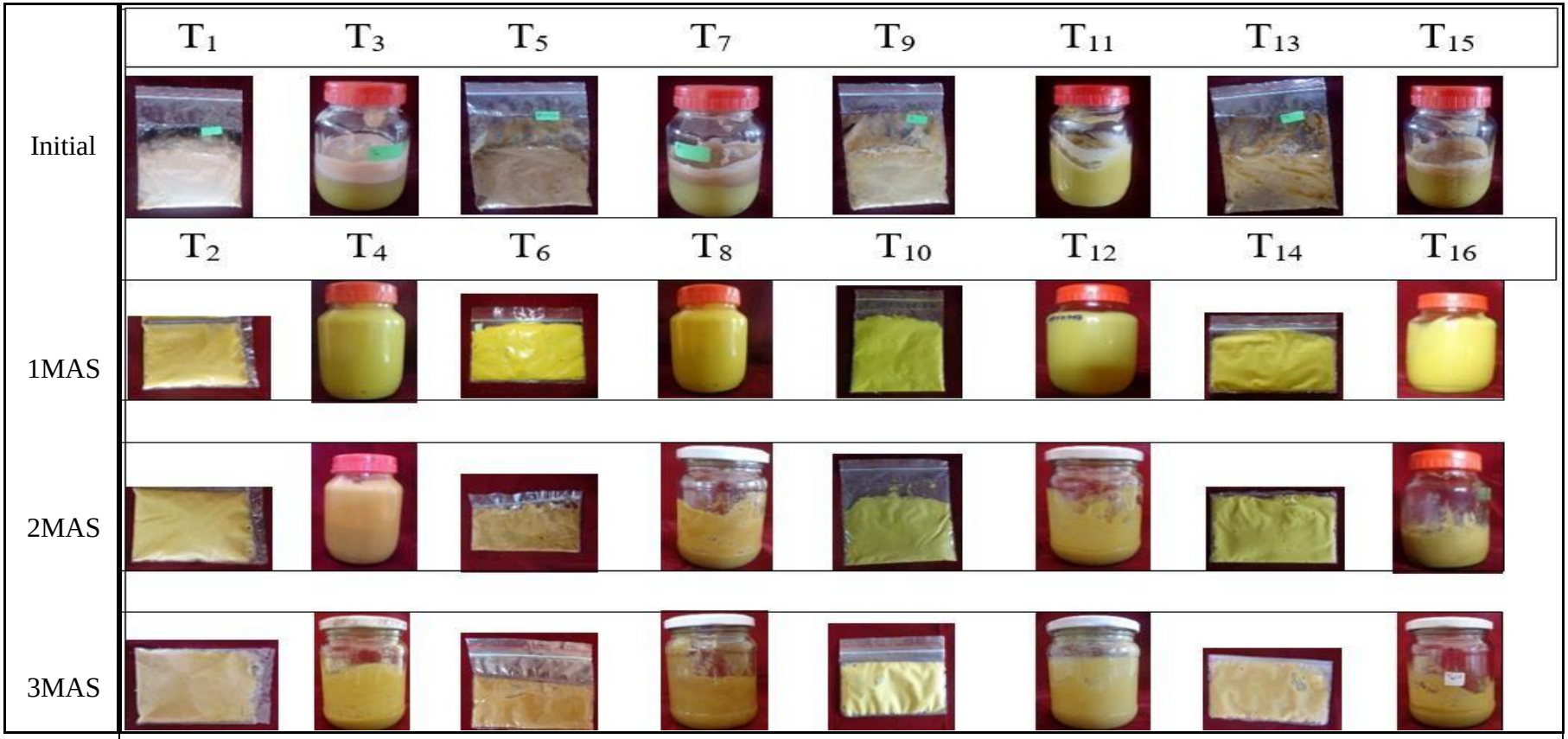
4.4.1. Process standardization for preparation and storage of avocado pulp

Avocado fruit pulp pre-treated with 0.5 % citric acid and ascorbic acid separately, in combination with a preservative, either potassium metabisulphite (KMS) or sodium benzoate @ 0.1 %, enclosed in glass jars and in vacuum packed LDPE (200 gauge) bags separately and stored under ambient and refrigerated conditions for 3 months are shown in Plate 13.

4.4.1.1. Pulp yield

The average pulp yield obtained from ripe avocado fruits after removing the peel and seed was 71.42 %.

Plate 13. Pre-treated avocado fruit pulp during storage



T_1 - Pulp + CA +KMS + LDPE bags + AT
T_2 - Pulp + CA +KMS + LDPE bags + RT
T_3- Pulp + CA + KMS + glass jars + AT
T_4 - Pulp + CA +KMS + glass jars + RT
T_5- Pulp + CA +NaB + LDPE bags + AT
T_6 - Pulp + CA+NaB + LDPE bags + RT
T_7 - Pulp + CA +NaB + glass jars + AT
T_8- Pulp + CA +NaB + glass jars + RT
T_9 -Pulp + AA+KMS + LDPE bags + AT
T_{10} - Pulp + AA +KMS+ LDPE bags+ RT
T_{11}- Pulp + AA +KMS+ glass jars + AT
T_{12}- Pulp + AA +KMS + glass jars + RT
T_{13} - Pulp + AA +NaB + LDPE bags+ AT
T_{14}- Pulp + AA +NaB + LDPE bags + RT
T_{15}- Pulp + AA +NaB + glass jars + AT
T_{16} - Pulp + AA +NaB + glass jars + RT
MAS: Months after storage

It was observed that avocado fruit pulp stored under ambient condition T_1 (fruit pulp pre-treated with citric acid and KMS packed in LDPE bags and stored in ambient temperature), T_3 (fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in ambient temperature), T_5 (fruit pulp pre-treated with citric acid and sodium benzoate packed in LDPE bags and stored in ambient temperature), T_7 (fruit pulp pre-treated with citric acid and sodium benzoate packed in glass jars and stored in ambient temperature), T_9 (fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in ambient temperature), T_{11} (fruit pulp pretreated with ascorbic acid and KMS packed in glass jars and stored in ambient temperature), T_{13} (fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in ambient temperature) and T_{15} (fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in glass jars and stored in ambient temperature) became unmarketable within 1 month after storage, regardless of the different pre-treatments and packaging conditions. In these treatments fruit pulp discoloration, rancid taste, off flavour development and loss of texture along with microbial spoilage were observed within one month of storage. The results revealed faster rate of oxidation and further deterioration of avocado fruit pulp due to the exposure to light and air, under ambient condition.

4.4.1.2. Total Soluble Solids (ºBrix)

Total soluble solids of avocado fruit pulp decreased during storage of three months among which significantly higher value was observed in fruit pulp pre-treated with citric acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_6) (7.60 ºBrix) one month after storage and it varied non significantly after two months of storage (Table 16a).

4.4.1.3. Titratable acidity (%)

Significantly higher titratable acidity was observed in T_6 (fruit pulp pre-treated with citric acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature), T_8 (fruit pulp pre-treated with citric acid and sodium benzoate packed in glass jars and stored in refrigerated temperature), T_{10} (fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature) and T_{14} (fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature) (0.67 %) after one month of storage and after two months of storage it varied without any significance difference among the

treatments. In all the treatments, titratable acidity increased after one month of storage except T_4 (fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature), which decreased to significantly lower titratable acidity (0.22%) (Table 16a).

4.4.1.4. Total protein (g/100g)

It was observed that during storage total protein content of avocado pulp increased one month after storage and decreased after two months of storage. After 1 and 2 months of storage, T_{12} (fruit pulp treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) recorded significantly higher protein content of 3.56 and 1.36 g/100g, respectively. An increase in the total protein content (1.55 g/100g) was observed in T_4 (fruit pulp treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature) and T_{10} (fruit pulp treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature) after three months of storage (Table 16a).

4.4.1.5. Vitamin C (mg/100g)

Vitamin C content in avocado fruit pulp increased from an initial value of 12.00 mg/100g in all the treatments after one month of storage and decreased thereafter towards the end of storage and it remained non-significant among the treatment throughout the storage (Table 16a).

4.4.1.6 Total phenols (mg/100g)

Total phenolic content varied significantly among the treatments only after 2 months of storage and significantly higher value was noticed in fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature (T_{12}) (18.83 mg/100g) as per the result shown in Table 16b.

A steep rise in the total phenol content was observed after one month of storage and it decreased after 2 months and again increased after three months of storage in the remaining treatments such as T_4 (fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature) (18.33 mg/100g), T_{10} (fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature) (53.33 mg/100g) and T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) (51.67 mg/100g).

Table 16a. Effect of food additives on quality of avocado pulp during storage

Treatments	TSS (^{0} Brix)				Titratable acidity (%)				Total protein (g/100g)				Vitamin C (mg/100g)			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_2	7.53	7.00 ^{ab}	4.50		0.32	0.67^{a}	0.67	*	0.287	2.39^{d}	1.00^{bc}	*	12.00	24.00	12.00	*
T_4		6.40^{abc}	5.40	3.73		0.22^{c}	0.56	1.12		2.87^{cd}	1.12^{ab}	1.55		18.67	15.00	8.00
T_6		7.60^{a}	*	*		0.67^{a}	*	*		3.00^{bc}	*	*		18.67	*	*
T_8		5.40^{c}	*	*		0.67^{a}	*	*		3.30^{abc}	*	*		21.33	*	*
T_{10}		6.50^{abc}	3.00	2.13		0.67^{a}	0.56	0.61		2.91$^{bc d}$	1.03^{bc}	1.55		15.33	14.00	8.00
T_{12}		5.93^{bc}	2.47	1.67		0.45^{b}	0.45	0.28		3.56^{a}	1.36^{a}	1.27		17.33	14.00	8.00
T_{14}		7.00 ^{ab}	2.60	*		0.67^{a}	0.34	*		3.38^{b}	0.91^{c}	*		18.67	15.00	*
T_{16}		6.73a^{b}	3.00	*		0.34^{bc}	0.78	*		2.88^{cd}	1.03^{c}	*		18.67	15.00	*
CD (0.05)		**1.21**	**NS**			**0.13**	**NS**			**5.49**	**2.032**			**NS**	**NS**	

T_1 - Pulp + CA +KMS+ LDPE bags+ AT T_2 - Pulp + CA +KMS + LDPE bags + RT T_3 - Pulp + CA +KMS + glass jars + AT
T_4 - Pulp + CA +KMS+ glass jars + RT T_5 - Pulp + CA +NaB + LDPE bags + AT T_6 - Pulp + CA +NaB + LDPE bags + RT
T_7 - Pulp + CA +NaB + glass jars + AT T_8 - Pulp + CA +NaB + glass jars + RT T_9 - Pulp + AA +KMS + LDPE bags + AT
T_{10}- Pulp + AA +KMS + LDPE bags+ RT T_{11}- Pulp + AA +KMS + glass jars + AT T_{12}- Pulp + AA +KMS + glass jars + RT
T_{13}- Pulp + AA +NaB + LDPE bags+ AT T_{14}- Pulp + AA +NaB + LDPE bags + RT T_{15}- Pulp + AA +NaB + glass jars + AT
T_{16}- Pulp + AA +NaB + glass jars + RT
MAS: Months after storage * - Unmarketable NS- Non-significant

4.4.1.7. Total carbohydrate (g/100g)

Total carbohydrate present in the fruit pulp before storage was 0.93 g/100g and it varied non-significantly among the treatments after one month of storage. Significantly higher value of total carbohydrate was observed in T_4 (fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature) (8.87 g/100g) and T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) (8.83 g/100g) after 2 months of storage. It was observed that total carbohydrate content increased after one month of storage and decreased afterwards up to the end of storage period (Table 16b).

4.4.1.8. Total fat (%)

From an initial value of 1.51 %, total fat content increased gradually during storage and varied significantly among the treatments and higher values were observed in fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature (T_{10}) (14.10 %) and fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_{14}) (18.85 %) after 1 and 2 months of storage, respectively (Table 16b).

4.4.1.9. Water activity

It was observed that as the storage period increased, water activity in the samples also increased and remained without much difference towards the end of storage (Table 16b). A significant variation was noticed only after one month of storage, among which lower water activity (0.980) was seen in fruit pulp pre-treated with citric acid and KMS, packed in LDPE bags and stored in refrigerated temperature (T_2) and fruit pulp pre-treated with ascorbic acid and KMS, packed in LDPE bags and stored in refrigerated temperature (T_{10}).

4.4.1.10. Peroxide value (meq/kg)

Peroxide value of avocado fruit pulp increased throughout the storage of three months, from an initial value of 1.39 meq/kg (Table 16c). After one month of storage, peroxide value varied significantly among the treatments in which the lower value (1.53 meq /kg) was observed in fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature (T_4) and highest (5.50 meq/kg) in fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in

Table 16b. Effect of food additives on quality of avocado pulp during storage

Treatments	Total phenols (mg/100g)				Total carbohydrate (g/100g)				Total fat (%)				Water activity			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_2	10.67	49.33	10.67 c	*	0.93	12.43	6.43^{b}	*	1.51	7.90^{e}	10.18^{c}	*	0.970	0.980^{c}	0.990	*
T_4		55.50	14.00 b	18.33		9.07	8.87^{a}	1.33		11.43^{c}	16.31^{b}	18.22		0.982^{c}	0.990	0.986
T_6		49.50	*	*		4.90	*	*		6.52^{f}	*	*		0.995^{a}	*	*
T_8		57.83	*	*		4.47	*	*		4.40^{g}	*	*		0.998^{a}	*	*
T_{10}		37.43	17.17 a	53.33		5.57	6.17^{b}	1.03		14.10^{a}	15.50^{b}	17.18		0.980^{c}	0.990	0.991
T_{12}		47.83	18.83 a	51.67		8.17	8.83^{a}	1.53		12.46^{b}	9.30^{c}	13.82		0.993^{b}	0.990	0.990
T_{14}		56.00	16.83 a	*		7.50	5.90^{b}	*		10.13^{d}	18.85^{a}	*		0.990^{b}	0.990	*
T_{16}		62.83	16.67 a	*		11.37	6.40^{b}	*		2.31^{h}	7.27^{d}	*		0.991^{b}	0.990	*
CD (0.05)		**NS**	**2.37**			**NS**	**2.30**			**0.56**	**1.62**			**0.01**	**NS**	

T_1 - Pulp + CA +KMS+ LDPE bags+ AT T_2 - Pulp + CA +KMS + LDPE bags + RT T_3 - Pulp + CA +KMS + glass jars + AT
T_4 - Pulp + CA +KMS+ glass jars + RT T_5 - Pulp + CA +NaB + LDPE bags + AT T_6 - Pulp + CA +NaB + LDPE bags + RT
T_7 - Pulp + CA +NaB + glass jars + AT T_8 - Pulp + CA +NaB + glass jars + RT T_9 - Pulp + AA +KMS + LDPE bags + AT
T_{10}- Pulp + AA +KMS + LDPE bags+ RT T_{11}- Pulp + AA +KMS + glass jars + AT T_{12}- Pulp + AA +KMS + glass jars + RT
T_{13}- Pulp + AA +NaB + LDPE bags+ AT T_{14}- Pulp + AA +NaB + LDPE bags + RT T_{15}- Pulp + AA +NaB + glass jars + AT
T_{16}- Pulp + AA +NaB + glass jars + RT

MAS: Months after storage * - Unmarketable NS- Non-significant

refrigerated temperature (T_{14}). It varied non-significantly among the treatments after two months of storage.

4.4.1.11. Viscosity (cP)

Viscosity decreased throughout the storage with an initial viscosity of 3276 cP and fruit pulp pre-treated with ascorbic acid and potassium metabisulphite, packed in LDPE bags and stored in refrigerated temperature (T_{10}) was recorded with significantly higher viscosity of 2505.6 and 846 cP after one and two months of storage, respectively. It was observed that the consistency of fruit pulp decreased and became more watery towards the end of storage, which may be due to the decrease in viscosity (Table 16c).

4.4.1.12. Polyphenol oxidase activity (unit/ml)

Polyphenol oxidase activity increase during storage and significantly lower enzyme activity was observed in fruit pulp pre-treated with citric acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_6) and all the samples pre-treated with ascorbic acid after one month of storage. After two months of storage, significantly lower enzyme activity was observed in fruit pulp treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_{14}), which indicated the lower rate of browning in fruit pulp (Table 16c).

4.4.1.13. Microbial load (bacteria, fungi and yeast)

Among the avocado fruit pulp stored under refrigeration, microbial population was very less up to 1 month of storage and it increased towards second and third months after storage, in which yeast and bacterial population were higher than the fungal population (Table 17.). In the fruit pulp before storage, bacterial population was 0.66 x 10^5 cfu/g. After one month of storage, microbial population were not observed in the samples except bacterial count in T_6 (fruit pulp pre-treated with citric acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature) (1 x 10^5 cfu/g), T_{10} (fruit pulp pre-treated with ascorbic acid and KMS, packed in LDPE bags and stored in refrigerated temperature) (1 x 10^5 cfu/g) and T_{14} (fruit pulp pre-treated with ascorbic acid and sodium benzoate, packed in LDPE bags and stored in refrigerated temperature) (7 x 10^5 cfu/g) and fungal count in T_{14} (fruit pulp pre-treated with ascorbic acid and sodium benzoate, packed in LDPE bags and stored in refrigerated temperature) (2 x 10^3 cfu/g). Microbial count was nil in T_{16} (fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in glass jars and stored in refrigerated temperature) up to 2

Table 16c. Effect of food additives on quality of avocado pulp during storage

Treatments	Peroxide value (meq./kg)				Viscosity (cP)				Polyphenol oxidase activity (unit/ml)			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_2	1.39	3.91^{bc}	15.71	*	3276.0	2160.20^{b}	545.86^{b}	*	150.00	188.33^{ab}	473.33^{a}	*
T_4		1.53^{e}	16.56	23.15		1710.60^{c}	376.30^{c}	52.50		125.00^{b}	45.00^{c}	461.67
T_6		2.22^{de}	*	*		1611.3^{cd}	*	*		10.00^{c}	66.67^{c}	*
T_8		2.30^{de}	*	*		1513.90^{cd}	*	*		300.00^{a}	*	*
T_{10}		5.24^{ab}	17.20	26.59		2505.60^{a}	846.00^{a}	25.26		23.33^{c}	66.67^{c}	15.00
T_{12}		3.44^{cd}	19.20	35.85		1471.00^{d}	750.00^{a}	48.00		16.67^{c}	285.00^{b}	81.67
T_{14}		5.50^{a}	17.56	*		2158.30^{b}	232.30^{d}	*		11.67^{c}	18.33^{c}	*
T_{16}		2.08^{de}	33.51	*		2022.10^{b}	520.00^{bc}	*		16.67^{c}	478.33^{a}	*
CD (0.05)		**1.41**	**NS**			**238.60**	**163.26**			**113.043**	**121.615**	**NS**

T_1 - Pulp + CA +KMS+ LDPE bags+ AT
T_2 - Pulp + CA +KMS + LDPE bags + RT
T_3 - Pulp + CA +KMS + glass jars + AT
T_4 - Pulp + CA +KMS+ glass jars + RT
T_5 - Pulp + CA +NaB + LDPE bags + AT
T_6 - Pulp + CA +NaB + LDPE bags + RT
T_7 - Pulp + CA +NaB + glass jars + AT
T_8 - Pulp + CA +NaB + glass jars + RT
T_9 - Pulp + AA +KMS + LDPE bags + AT
T_{10}- Pulp + AA +KMS + LDPE bags+ RT
T_{11}- Pulp + AA +KMS + glass jars + AT
T_{12}- Pulp + AA +KMS + glass jars + RT
T_{13}- Pulp + AA +NaB + LDPE bags+ AT
T_{14}- Pulp + AA +NaB + LDPE bags + RT
T_{15}- Pulp + AA +NaB + glass jars + AT
T_{16}- Pulp + AA +NaB + glass jars + RT
MAS: Months after storage
* - Unmarketable
NS- Non-significant

months after storage. After 3 months of storage, lower microbial count was observed in T_{10} (fruit pulp pre-treated with ascorbic acid and KMS, packed in LDPE bags and stored in refrigerated temperature) with 9.33 x 10^5 cfu/g bacterial colonies, 11.33 x 10^4 cfu/g yeast colonies and 1.50 x 10^3 cfu/g fungal colonies.

4.4.1.14 Organoleptic evaluation

At the beginning of storage, organoleptic scores for all the sensory attributes not varied among the treatments except the lower values of after taste in fruit pulp pre-treated with citric acid. During the storage period, higher scores for most of the sensory attributes were observed in T_{10} (fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature) and T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) (Table 18a and 18b).

During storage, T_{10} (fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature) had comparatively high total organoleptic scores at the beginning (62.2) and three (38.8) months after storage. After one (55.7) and two (46.1) months of storage, T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) had higher total organoleptic scores. After 1 and 2 months of storage, in all treated samples of fruit pulp, flavour, odour, taste and after taste had only lower scores due to off flavour development and rancidity, while appearance, colour and texture had higher scores. After one month of storage, higher scores were recorded for colour (56) and appearance (54) and after two months of storage, it was for texture (37) followed by colour (35) and appearance (35). After three months of storage, among treatments such as T_4 (fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature) (35.1), T_{10} (fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature) (38.8) and T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) (36), higher scores were recorded for appearance (19) and colour (18), in which browning were also observed.

4.4.1.15. Cost of Production

The cost required for the preparation of 1 kg avocado pulp added with 0.5 % ascorbic acid, 0.1 % KMS, packed in LDPE pouches or glass jars stored in refrigeration for one month was about Rs. 339.98/- (Table 19Fig).

Table 17. Effect of food additives on microbial population of avocado pulp during storage

Treatments	Initial			1MAS			2MAS			3MAS		
	F	Y	B	F	Y	B	F	Y	B	F	Y	B
T_2	0	0	0.66	0	0	0	1.00^b	0	0	*	*	*
T_4				0	0	0	1.00^b	44.33^a	23.00^a	1.50^b	46.33^a	30.67^a
T_6				0	0	1.00^b	*	*	*	*	*	*
T_8				0	0	0	*	*	*	*	*	*
T_{10}				0	0	1.00^b	1.00^b	9.33^b	7.00^b	1.50^b	11.33^b	9.33^b
T_{12}				0	0	0	0	12.00^b	5.33^b	7.00^a	14.33^b	12.67^b
T_{14}				2.00	0	7.00^a	5.67^a	9.00^b	10.33^b	*	*	*
T_{16}				0	0	0	0	0	0	*	*	*
CD (0.05)				**NS**	**NS**	**2.90**	**1.96**	**10.03**	**13.31**	**2.25**	**6.31**	**13.20**

T_1 - Pulp + CA +KMS+ LDPE bags+ AT T_2 - Pulp + CA +KMS + LDPE bags + RT T_3 - Pulp + CA +KMS + glass jars + AT
T_4 - Pulp + CA +KMS+ glass jars + RT T_5 - Pulp + CA +NaB + LDPE bags + AT T_6 - Pulp + CA +NaB + LDPE bags + RT
T_7 - Pulp + CA +NaB + glass jars + AT T_8 - Pulp + CA +NaB + glass jars + RT T_9 - Pulp + AA +KMS + LDPE bags + AT
T_{10}- Pulp + AA +KMS + LDPE bags+ RT T_{11}- Pulp + AA +KMS + glass jars + AT T_{12}- Pulp + AA +KMS + glass jars + RT
T_{13}- Pulp + AA +NaB + LDPE bags+ AT T_{14}- Pulp + AA +NaB + LDPE bags + RT T_{15}- Pulp + AA +NaB + glass jars + AT
T_{16}- Pulp + AA +NaB + glass jars + RT

MAS: Months after storage * - Unmarketable NS- Non-significant

B: Bacteria (10^5 cfu/ml) Y: Yeast (10^4 cfu/ml) F: Fungi (10^3 cfu/ml)

Table 18a. Effect of food additives on organoleptic scores of avocado pulp (Initial and one month after storage)

Treatments	Initial									1MAS								
	A	**C**	**TE**	**O**	**F**	**TA**	**AT**	**OA**	**TS**	**A**	**C**	**TE**	**O**	**F**	**TA**	**AT**	**OA**	**TS**
T_2	7.7	7.7	7	6.5	6.9	4.7	4.4	6.5	**51.4**	7.1	7.0	6.5	6.6	4.6	5.1	5.4	6.1	**48.4**
T_4	7.5	6.9	6.7	6.7	6.6	4.8	4.3	6.4	**49.9**	7.0	6.3	6.4	5.9	4.3	6.1	5.5	5.8	**47.3**
T_6	7.3	6.7	6.8	6.4	6.4	4.6	4.8	6.7	**49.7**	6.4	6.3	7.5	6.0	4.6	5.1	4.0	6.2	**46.1**
T_8	7.3	6.9	6.5	6.8	6.6	4.8	3.5	7.2	**49.6**	6.7	6.4	7.2	5.5	4.9	3.3	4.1	6.2	**44.3**
T_{10}	8.3	7.1	7.8	7.1	7.2	5.5	6.4	7.6	**57**	7.4	7.2	7.7	5.5	6.0	5.2	6.0	6.2	**51.2**
T_{12}	8.0	7.1	7.5	7.6	7.0	4.3	6.7	7.4	**55.6**	7.4	7.1	7.8	7.4	6.4	6.1	6.7	6.8	**55.7**
T_{14}	8.0	6.9	6.6	7.2	6.8	5.0	6.1	7.7	**54.3**	7.7	6.8	7.5	5.8	4.9	4.2	5.1	6.6	**48.6**
T_{16}	8.1	6.8	7.4	6.6	6.7	5.9	6.4	7.5	**55.4**	7.9	7.0	7.0	5.9	5.0	4.6	6.3	6.8	**50.5**
Kendall's W	**0.18**	**0.11**	**0.19**	**0.16**	**0.04**	**0.19**	**0.62**	**0.28**		**0.28**	**0.25**	**0.22**	**0.19**	**0.38**	**0.30**	**0.46**	**0.13**	

T_1 - Pulp + CA +KMS+ LDPE bags+ AT T_2 - Pulp + CA +KMS + LDPE bags + RT T_3 - Pulp + CA +KMS + glass jars + AT
T_4 - Pulp + CA +KMS+ glass jars + RT T_5 - Pulp + CA +NaB + LDPE bags + AT T_6 - Pulp + CA +NaB + LDPE bags + RT
T_7 - Pulp + CA +NaB + glass jars + AT T_8 - Pulp + CA +NaB + glass jars + RT T_9 - Pulp + AA +KMS + LDPE bags + AT
T_{10}- Pulp + AA +KMS + LDPE bags+ RT T_{11}- Pulp + AA +KMS + glass jars + AT T_{12}- Pulp + AA +KMS + glass jars + RT
T_{13}- Pulp + AA +NaB + LDPE bags+ AT T_{14}- Pulp + AA +NaB + LDPE bags + RT T_{15}- Pulp + AA +NaB + glass jars + AT
T_{16}- Pulp + AA +NaB + glass jars + RT

MAS: Months after storage * - Unmarketable NS- Non-significant
A: Appearance C: Colour Te: Texture F: Flavour Ta: Taste At: After taste
O: Odour Oa: Overall acceptability Ts: Total score

Table 18b. Effect of food additives on organoleptic scores of avocado pulp 2 and 3 months after storage

Treatments	2MAS									3MAS								
	A	**C**	**TE**	**O**	**F**	**TA**	**AT**	**OA**	**TS**	**A**	**C**	**TE**	**O**	**F**	**TA**	**AT**	**OA**	**TS**
T_2	4.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	**43**	*	*	*	*	*	*	*	*	*
T_4	5.9	5.6	5.3	4	3.9	4.3	4.1	5.9	**39**	4.3	6.3	6.9	3.1	3.0	3.2	3.1	5.2	**35.1**
T_6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
T_8	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
T_{10}	6.7	6.7	6	3.6	3.4	3.9	3.3	5.1	**38.7**	6	7.2	6.7	3.7	3.6	3.9	2.7	5.0	**38.8**
T_{12}	6.6	6.8	6.5	4.8	5.1	4.8	5	6.5	**46.1**	5.8	6.3	6.1	3.2	3.5	3.8	2.7	4.6	**36.0**
T_{14}	5.6	7	6.2	4.5	4.1	5.1	6.7	6.5	**45.7**	*	*	*	*	*	*	*	*	*
T_{16}	6.5	5.7	7.2	4	3.5	3.1	3.7	5.3	**39**	*	*	*	*	*	*	*	*	*
Kendall's W	**0.29**	**0.22**	**0.33**	**0.04**	**0.34**	**0.24**	**0.51**	**0.24**		**0.48**	**0.22**	**0.27**	**0.11**	**0.08**	**0.15**	**0.17**	**0.04**	

T_1 - Pulp + CA +KMS+ LDPE bags+ AT T_2 - Pulp + CA +KMS + LDPE bags + RT T_3 - Pulp + CA +KMS + glass jars + AT
T_4 - Pulp + CA +KMS+ glass jars + RT T_5 - Pulp + CA +NaB + LDPE bags + AT T_6 - Pulp + CA +NaB + LDPE bags + RT
T_7 - Pulp + CA +NaB + glass jars + AT T_8 - Pulp + CA +NaB + glass jars + RT T_9 - Pulp + AA +KMS + LDPE bags + AT
T_{10}- Pulp + AA +KMS + LDPE bags+ RT T_{11}- Pulp + AA +KMS + glass jars + AT T_{12}- Pulp + AA +KMS + glass jars + RT
T_{13}- Pulp + AA +NaB + LDPE bags+ AT T_{14}- Pulp + AA +NaB + LDPE bags + RT T_{15}- Pulp + AA +NaB + glass jars + AT
T_{16}- Pulp + AA +NaB + glass jars + RT

MAS: Months after storage * - Unmarketable NS- Non-significant
A: Appearance C: Colour Te: Texture F: Flavour Ta: Taste At: After taste
O: Odour Oa: Overall acceptability Ts: Total score

Table 19. Cost of production of 1 kg avocado pulp

Avocado pulp	Rate	Quantity	Cost (Rs.)
Avocado fruits	Rs.200/kg	1.25 kg	250.00
Ozonisation (2 ppm for 15 min.)	Rs.5/unit	1.7 Kwh/kg O_3	1.00
Ascorbic acid (0.5 %)	Rs.300/500 g	5 g	3.00
KMS (0.1 %)	Rs.368/500 g	1 g	0.73
Glass jars	Rs. 10/bottle	4 no.	40.00
LDPE (200 gauge) pouches	Rs. 100/kg	4 no.	4.00
Vacuum packing (1kWh)	Rs.5/unit	15 min	1.25
Labour charge	Rs.600/8 hr	2 hr	40.00
Total			**Rs.339.98/kg**

Considering the longer shelf life of three months, higher total protein (T_{12}: 3.56 to 1.36 g/100g), total phenols (T_{12}: 18.83 mg/100g), total carbohydrate (T_{12}: 8.83 g/100g), total fat (T_{10}: 14.10%), viscosity (T_{10}: 2505.6 and 846.0) and organoleptic scores along with lower water activity (T_{10}: 0.980), polyphenol oxidase activity (T_{10}: 23.33 and 66.67 and T_{12}: 16.67), microbial population during storage, avocado fruit pulp treated with ascorbic acid and KMS packed in both LDPE bags (T_{10}) and glass jars (T_{12}) stored under refrigeration were observed as the ideal pretreatments for the preservation of avocado fruit pulp.

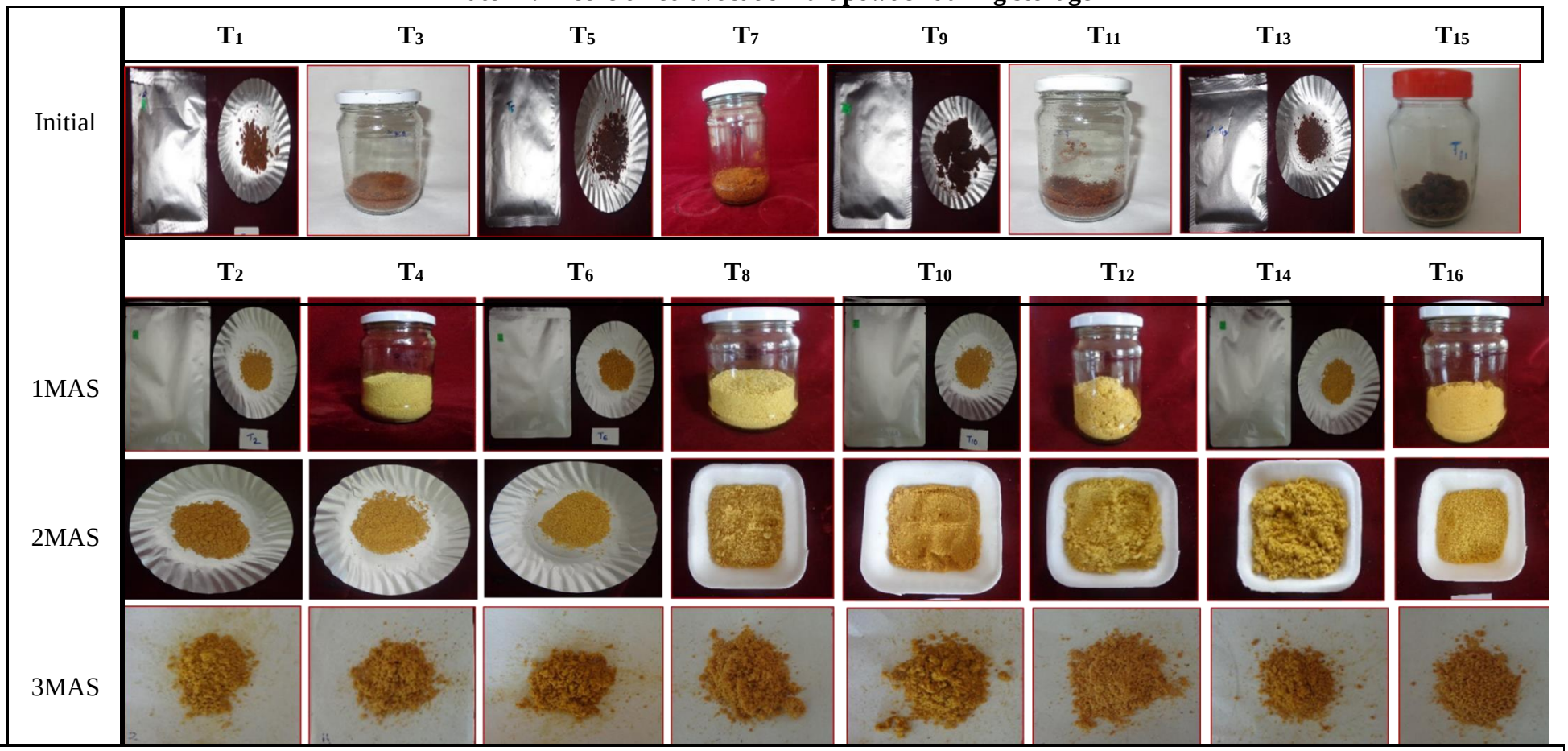
4.4.2. Optimization of process conditions for preparation of avocado fruit powder

Freeze dried avocado powder pre-treated with maltodextrin at 1 to 5 % concentration, packed in LDPE laminated aluminium pouches and in glass jars stored under ambient and refrigerated conditions for 3 months are shown in Plate 14.

Yield ratio of freeze dried powder to the fruit pulp was 38.57 %.

It was observed that freeze dried avocado fruit powder stored under ambient condition T_1 (fruit pulp pre-treated with 2 % maltodextrin packed in LDPE bags and stored in ambient temperature), T_3 (fruit pulp pre-treated with 2 % maltodextrin packed in glass jars and stored in ambient temperature), T_5 (fruit pulp pre-treated with 3 % maltodextrin packed in LDPE bags and stored in ambient temperature), T_7 (fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in ambient temperature), T_9 (fruit pulp pre-treated with 4 % maltodextrin packed in LDPE bags

Plate 14. Freeze dried avocado fruit powder during storage



T_1 - Pulp + MD (2%) +LDPE bags +AT T_2 - Pulp + MD (2%) +LDPE bags +RT T_3 - Pulp + MD (2%) +glass jars +AT T_4 - Pulp + MD (2%) +glass jars +RT
T_5 - Pulp + MD (3%) +LDPE bags +AT T_6 - Pulp + MD (3%) +LDPE bags +RT T_7 - Pulp + MD (3%) +glass jars +AT T_8 - Pulp + MD (3%) +glass jars +RT
T_9 - Pulp + MD (4%) + LDPE bags +AT T_{10}- Pulp + MD (4%) + LDPE bags +RT T_{11}- Pulp + MD (4%) + glass jars +AT T_{12} - Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT T_{14}- Pulp + MD (5%) + LDPE bags +RT T_{15}- Pulp + MD (5%) + glass jars +AT T_{16}- Pulp + MD (5%) + glass jars +RT
MAS: Months after storage

and stored in ambient temperature), T_{11} (fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in ambient temperature), T_{13} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in ambient temperature) and T_{15} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in ambient temperature) were unmarketable within 1 month after storage, regardless of the different pre-treatments and packaging conditions. In these treatments, fruit powder discoloration, rancid taste, off flavour development and loss of texture were observed within one month of storage. The results may attributed to the faster rate of oxidation and further deterioration of avocado fruit powder due to the exposure of light, air and atmospheric moisture under ambient condition.

4.4.2.1. Bulk density (g/cm^3)

It was observed that bulk density decreased during storage in all the treatments of freeze dried avocado fruit powder. It was observed that bulk density increased with the increasing concentration of high molecular weight additives like maltodextrin due to its bulking properties. Significantly higher values of bulk density were recorded as 0.45 g/cm^3 in T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) after 1 month, 0.26 and 0.27 g/cm^3 in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) and T_{10} (fruit pulp pre-treated with 4 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) after 2 months of storage and 0.23 g/cm^3 in T_2 (fruit pulp pre-treated with 2 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) and T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) after 3 months, respectively (Table 20).

4.4.2.2. Solubility (%)

During storage, solubility of powder increased after one month of storage and decreased towards the end of storage. Solubility varied non-significantly among the treatments after one month of storage. Significantly higher solubility of 65.03 % and 57.66 % were observed in T12 (fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in refrigerated temperature) and T8 (fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in refrigerated temperature) after two and three months of storage, respectively. Higher solubility was observed in fruit

powder pre-treated with higher maltodextrin content (Table 20) indicating the better reconstitution of powder.

4.4.2.3. Hygroscopicity (%)

A gradual rise was seen in the hygroscopicity of powder during three months of storage (Table 20). Lower hygroscopicity was recorded in samples pre-treated with higher concentration on maltodextrin. Significantly lower values were observed in T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) after one (0.25 %) and two (0.33 %) months of storage and in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) after three (1.31 %) months of storage.

4.4.2.4. Colour values (L, a, b)

L value decreased towards the end of storage, however retention of higher values throughout the storage were observed T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature), T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature), T_{12} (fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in ambient temperature) and T_{10} (fruit pulp pre-treated with 4 % maltodextrin packed in LDPE bags and stored in ambient temperature) with comparatively higher maltodextrin content which indicated the luminosity or brightness of powder with the addition of maltodextrin (Table 21).

A gradual increase was observed in a* value of colour which indicated the change in colour of powder from greenish to reddish colour. Significantly lower a* values (higher negative values) were observed in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) and T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) after 1 (-14.21 and -14.18) and 2 (-3.63) months of storage and in T_{12} (fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in ambient temperature) (-0.38) after 3 months indicating the retention of green colour of powder (Table 21). Colour value b* increased gradually up to 2 months, after which a steep rise was noticed up to end of storage period. Significantly lower b* value was noticed in T_{14} (fruit pulp pre- treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (25.30) after one month, in T_{12} (fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in refrigerated temperature) (40.28) after

Table 20. Effect of food additives on physical qualities of avocado fruit powder

Treatments	Bulk density (g/cm3)				Solubility (%)				Hygroscopicity (%)			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_2	0.29	0.36^{d}	0.23^{bc}	0.23^{a}	55.93	55.00	52.33^{ab}	29.63^{c}	0.54	0.41^{b}	0.68^{c}	2.27^{ab}
T_4		0.34^{e}	0.19^{e}	0.15^{d}		47.33	34.13^{bc}	24.23^{d}		0.69^{a}	0.93^{b}	2.38^{a}
T_6		0.44^{a}	0.24^{b}	0.18^{c}		46.33	32.47^{c}	27.86^{cd}		0.62^{a}	0.61^{cd}	1.81^{bc}
T_8		0.38^{c}	0.21^{d}	0.19^{bc}		61.00	59.00^{a}	57.66^{a}		0.69^{a}	0.85^{c}	2.14^{abc}
T_{10}		0.37^{cd}	0.27^{a}	0.19^{bc}		63.67	58.67^{a}	53.56^{ab}		0.42^{b}	1.05^{a}	1.71^{cd}
T_{12}		0.41^{b}	0.19^{e}	0.20^{abc}		84.07	65.033^{a}	50.00^{b}		0.33^{bc}	0.63^{cd}	1.94^{abc}
T_{14}		0.39^{c}	0.26^{a}	0.23^{a}		72.00	62.533^{a}	52.33^{b}		0.32^{bc}	0.55^{d}	1.31^{d}
T_{16}		0.45^{a}	0.22^{cd}	0.22^{ab}		76.33	63.333^{a}	51.00^{b}		0.25^{c}	0.33^{e}	1.80^{bc}
CD (0.05)		**0.02**	**0.01**	**0.03**		**NS**	**4.38**	**4.94**		**0.14**	**0.10**	**0.49**

T_1- Pulp + MD (2%) +LDPE bags +AT
T_2- Pulp + MD (2%) +LDPE bags +RT
T_3 - Pulp + MD (2%) +glass jars +AT
T_4- Pulp + MD (2%) +glass jars +RT
T_5- Pulp + MD (3%) +LDPE bags +AT
T_6- Pulp + MD (3%) +LDPE bags +RT
T_7- Pulp + MD (3%) +glass jars +AT
T_8- Pulp + MD (3%) +glass jars +RT
T_9 - Pulp + MD (4%) + LDPE bags +AT
T_{10}- Pulp + MD (4%) + LDPE bags +RT
T_{11}- Pulp + MD (4%) + glass jars +AT
T_{12}- Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT
T_{14}- Pulp + MD (5%) + LDPE bags +RT
T_{15}- Pulp + MD (5%) + glass jars +AT
T_{16}- Pulp + MD (5%) + glass jars +RT
MAS: Months after storage
* - Unmarketable
NS- Non-significant

Table 21. Effect of food additives on colour value of avocado fruit powder

Treatments	Colour value- L				Colour value- a*				Colour value-b*			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_2	70.03	53.85^{e}	42.06^{c}	23.94^{f}	-19.66	-10.03^{f}	-1.93^{f}	0.05^{f}	24.99	29.90^{a}	41.78^{c}	94.54^{c}
T_4		57.45^{d}	48.60^{b}	26.46^{de}		-11.06^{e}	-1.96^{f}	0.02^{ef}		29.23^{ab}	41.93^{ab}	97.31^{a}
T_6		61.97^{c}	43.80^{c}	29.82^{b}		-11.07^{e}	-2.17^{e}	0.00^{de}		29.73^{a}	42.09^{a}	97.47^{a}
T_8		69.35^{b}	52.20^{ab}	25.68^{e}		-12.07^{c}	-2.56^{d}	0.04^{d}		28.33^{ab}	41.81^{b}	94.29^{c}
T_{10}		61.90^{c}	53.28^{a}	27.66^{cd}		-11.12^{d}	-3.29^{b}	-0.04^{c}		25.70^{c}	41.92^{ab}	96.57^{b}
T_{12}		76.64^{a}	50.46^{ab}	42.60^{a}		-13.13^{b}	-3.08^{c}	-0.38^{a}		27.23^{bc}	40.28^{e}	89.69^{d}
T_{14}		75.98^{a}	52.32^{ab}	29.58^{b}		-14.21^{a}	-3.63^{a}	-0.10^{b}		25.30^{c}	40.00^{f}	97.62^{a}
T_{16}		76.16^{a}	52.14^{ab}	28.02^{c}		-14.18^{a}	-3.63^{a}	-0.08^{b}		29.20^{ab}	40.87^{d}	86.46^{e}
CD (0.05)		**2.83**	**0.24**	**0.08**		**0.04**	**0.52**	**0.03**		**0.21**	**0.08**	**0.33**

T_1- Pulp + MD (2%) +LDPE bags +AT
T_2- Pulp + MD (2%) +LDPE bags +RT
T_3 - Pulp + MD (2%) +glass jars +AT
T_4- Pulp + MD (2%) +glass jars +RT
T_5- Pulp + MD (3%) +LDPE bags +AT
T_6- Pulp + MD (3%) +LDPE bags +RT
T_7- Pulp + MD (3%) +glass jars +AT
T_8- Pulp + MD (3%) +glass jars +RT
T_9 - Pulp + MD (4%) + LDPE bags +AT
T_{10}- Pulp + MD (4%) + LDPE bags +RT
T_{11}- Pulp + MD (4%) + glass jars +AT
T_{12}- Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT
T_{14}- Pulp + MD (5%) + LDPE bags +RT
T_{15}- Pulp + MD (5%) + glass jars +AT
T_{16}- Pulp + MD (5%) + glass jars +RT
MAS: Months after storage
* - Unmarketable
NS- Non-significant

2 months and in T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) (86.46) after 3 months of storage, which indicated comparatively lower yellowish colour of avocado powder.

4.4.2.5. Total Soluble Solids (ºBrix)

Total soluble solids of freeze dried avocado powder before storage was 4.25 ºBrix. Significantly higher TSS values were noticed in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (4.13 ºBrix**)** and T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) (7.70 ºBrix) after one and two months of storage, respectively. It was observed that TSS increased after two months of storage and thereafter decreased to the end of storage (Table 22a).

4.4.2.6. Titratable acidity (%)

Titratable acidity varied without any significant difference among the treatments throughout the storage. It was observed that initial content of titratable acidity in the freeze dried avocado powder was 5.36 %, which decreased after one month of storage and it increased after two months followed by a decreased after three months of storage (Table 22a).

4.4.2.7. Total protein (g/100g)

Total protein in the freeze dried avocado powder before storage was 9.60 g/100g, and it decreased during storage in all the treatments. It varied non-significantly among the treatments after one month of storage and significantly higher values were observed in T_8 (fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in refrigerated temperature) (6.24 g/100g) and T_{12} (fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in refrigerated temperature) (0.91 g/100g) after 2 and 3 months of storage, respectively (Table 22a).

4.4.2.8. Vitamin C (mg/100g)

A higher vitamin C content was noticed after one month of storage from an initial value of 15.73 mg/100g and thereafter it decreased. Significantly higher values were seen in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (70.00 mg/100g), T_{10} (fruit pulp pre-treated with 4 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (68.67 mg/100g) and T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and

stored in refrigerated temperature) (68.00 mg/100g) after one month of storage and in T_8 (fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in refrigerated temperature) (13.33 mg/100g) after three months of storage. It varied without any significance difference two months after storage (Table 22b).

4.4.2.9. Total phenols (mg/100g)

The initial total phenolic content of freeze dried avocado fruit powder was 41.98 mg/100g. It decreased in all the treatments except T_2 (fruit pulp pre-treated with 2 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (67.83 mg/100g) and T_{10} (fruit pulp pre-treated with 4 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (80.17 mg/100g) after one month of storage and increased thereafter to the end of storage. It varied without any significant difference among the treatments throughout the storage (Table 22b).

4.4.2.10. Total carbohydrate (g/100g)

Initial total carbohydrate content in freeze dried avocado fruit powder was 11.07 g/100g and it increased after one month of storage, varied non-significantly among the treatments (Table 22b). After two months of storage, it decreased in all the treatments and varied significantly with higher carbohydrate content in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (35.20 g/100g) and T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) (34.80 g/100g). After three months of storage, it decreased in all the samples except T_2 (fruit pulp pre-treated with 2 % maltodextrin packed in LDPE bags and stored in refrigerated temperature), T_4 (fruit pulp pre-treated with 2 % maltodextrin packed in glass jars and stored in refrigerated temperature), T_6 (fruit pulp pre-treated with 3 % maltodextrin packed in LDPE bags and stored in refrigerated temperature), and differed non-significantly among the treatments.

4.4.2.11. Total fat (%)

Total fat content of freeze dried avocado fruit powder decreased during storage from an initial value of 81.03 % (Table 22c). After one month of storage, significantly higher fat content of 58.31 %, 59.44 % and 60.43 % were seen in samples with higher concentration of maltodextrin such as T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature), T_{12} (fruit pulp pre-treated

Table 22a. Effect of food additives on biochemical qualities of avocado fruit powder

Treatments	TSS (^{0} Brix)				Titratable acidity (%)				Total protein (g/100g)			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_2	4.25	3.33^{d}	6.07^{c}	0.83	5.36	1.34	2.68	2.01	9.60	7.31	3.78^{c}	0.79^{c}
T_4		3.73^{b}	5.60^{d}	0.80		1.34	2.68	2.23		9.27	4.43^{bc}	0.84^{bc}
T_6		4.00^{a}	5.93^{c}	0.73		2.68	2.23	1.56		8.14	4.92^{bc}	0.87^{ab}
T_8		3.33^{d}	6.33^{b}	0.73		1.56	1.79	1.79		8.87	6.24^{a}	0.66^{d}
T_{10}		4.00^{a}	4.93^{e}	0.80		1.79	2.68	1.79		9.07	5.52^{ab}	0.81^{bc}
T_{12}		3.40^{cd}	5.00^{e}	0.70		1.79	2.23	2.01		8.14	4.20^{c}	0.91^{a}
T_{14}		4.13^{a}	6.07^{c}	0.73		1.79	2.68	1.79		8.00	4.35^{bc}	0.83^{bc}
T_{16}		3.60^{bc}	7.70^{a}	0.80		1.56	2.23	2.01		8.67	4.40^{bc}	0.82^{bc}
CD (0.05)		**0.26**	**0.22**	**NS**		**NS**	**NS**	**NS**		**NS**	**12.60**	**0.62**

T_1- Pulp + MD (2%) + LDPE bags +AT
T_2- Pulp + MD (2%) + LDPE bags +RT
T_3 - Pulp + MD (2%) + glass jars +AT
T_4- Pulp + MD (2%) + glass jars +RT
T_5- Pulp + MD (3%) + LDPE bags +AT
T_6- Pulp + MD (3%) + LDPE bags+RT
T_7- Pulp + MD (3%) + glass jars +AT
T_8- Pulp + MD (3%) + glass jars +RT
T_9 - Pulp + MD (4%) + LDPE bags+AT
T_{10}- Pulp + MD (4%) + LDPE bags +RT
T_{11}- Pulp + MD (4%) + glass jars +AT
T_{12}- Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT
T_{14}- Pulp + MD (5%) + LDPE bags +RT
T_{15}- Pulp + MD (5%) + glass jars +AT
T_{16}- Pulp + MD (5%) + glass jars +RT
MAS: Months after storage
* - Unmarketable
NS- Non-significant

Table 22b. Effect of food additives on biochemical qualities of avocado fruit powder

Treatments	Vitamin C (mg/100g)				Total phenols (mg/100g)				Total Carbohydrates (g/100g)			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_2	15.73	60.00^{bc}	11.07	7.07^{c}	41.98	67.83	78.33	117.33	11.07	26.13	12.67^{c}	22.67
T_4		55.33^{c}	9.33	9.47^{c}		34.33	39.33	107.33		28.67	10.40^{c}	16.53
T_6		43.33^{d}	7.60	12.93^{b}		35.17	37.00	103.33		36.20	14.64^{c}	19.33
T_8		64.00^{ab}	9.33	13.33^{a}		18.50	36.67	98.33		41.33	27.20^{b}	18.80
T_{10}		68.67^{a}	9.47	8.00^{c}		80.17	86.67	101.67		35.13	28.47^{ab}	20.20
T_{12}		64.67^{ab}	10.00	8.40^{c}		15.00	18.17	100.00		40.87	32.27^{ab}	16.31
T_{14}		70.00^{a}	21.33	9.07^{c}		19.83	34.67	98.33		38.53	35.20^{a}	20.27
T_{16}		68.00^{a}	10.80	9.60^{bc}		11.83	39.33	98.33		34.27	34.80^{a}	21.87
CD (0.05)		**6.28**	**NS**	**3.34**		**NS**	**NS**	**NS**		**NS**	**6.91**	**NS**

T_1- Pulp + MD (2%) + LDPE bags +AT T_2- Pulp + MD (2%) + LDPE bags +RT T_3 - Pulp + MD (2%) + glass jars +AT
T_4- Pulp + MD (2%) + glass jars +RT T_5- Pulp + MD (3%) + LDPE bags +AT T_6- Pulp + MD (3%) + LDPE bags+RT
T_7- Pulp + MD (3%) + glass jars +AT T_8- Pulp + MD (3%) + glass jars +RT T_9 - Pulp + MD (4%) + LDPE bags+AT
T_{10}- Pulp + MD (4%) + LDPE bags +RT T_{11}- Pulp + MD (4%) + glass jars +AT T_{12}- Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT T_{14}- Pulp + MD (5%) + LDPE bags +RT T_{15}- Pulp + MD (5%) + glass jars +AT
T_{16}- Pulp + MD (5%) + glass jars +RT

MAS: Months after storage * - Unmarketable NS- Non-significant

with 4 % maltodextrin packed in glass jars and stored in refrigerated temperature) and T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature), respectively and after two months of storage, it was T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) with 61.20 %. Total fat content varied non-significantly among the treatments after three months of storage.

4.4.2.12. Peroxide value (meq O_2/kg)

Peroxide value increased after one month of storage from an initial value of 2.77 meq O_2/kg and decreased after two months of storage and thereafter a steep increase was observed towards the end of storage, while peroxide value decreased with increase in concentration of maltodextrin (Table 22c). Lowest peroxide value after one month of storage was observed in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (1.44 meq O_2/kg), T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) (1.85 meq O_2/kg), T_{12} (fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in refrigerated temperature) (2.83 meq O_2/kg) and T_{10} (fruit pulp pre-treated with 4 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) (3.81 meq O_2/kg). After three months of storage, T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) recorded significantly lower peroxide value (5.31 meq O_2/kg), which indicated lower rate rancidity in the powder and it varied non-significantly after two months of storage.

4.4.2.13. Water activity

Water activity increased gradually during storage (Table 22c). Significantly lower values were noticed in samples with higher concentration of maltodextrin such as in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) and T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) after one month (0.37 and 0.36) and two months (0.55 and 0.54) and in T16 (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) (0.66) after three months of storage.

Table 22c. Effect of food additives on biochemical qualities of avocado fruit powder

Treatments	Total Fat (%)				Peroxide value (meq O_2/kg)				Water activity			
	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS	Initial	1MAS	2MAS	3MAS
T_2	81.03	39.43^{c}	28.83^{b}	28.38	2.77	6.49^{a}	6.08	21.49^{a}	0.46	0.48^{bc}	0.64^{a}	0.74^{a}
T_4		54.26^{b}	32.20^{b}	24.80		4.97^{b}	2.11	18.16^{ab}		0.44^{cd}	0.62^{ab}	0.70^{cd}
T_6		54.55^{b}	40.87^{b}	18.47		4.64^{bc}	3.54	17.86^{ab}		0.40^{de}	0.62^{a}	0.72^{b}
T_8		40.58^{c}	34.17^{b}	25.07		3.76^{c}	3.42	12.81^{bc}		0.42^{d}	0.58^{c}	0.70^{d}
T_{10}		53.65^{b}	44.07^{ab}	21.53		3.81^{d}	1.22	16.81^{ab}		0.53^{a}	0.60^{bc}	0.71^{bc}
T_{12}		59.44^{a}	44.43^{ab}	35.80		2.83^{d}	1.23	8.92^{cd}		0.50^{ab}	0.59^{c}	0.68^{e}
T_{14}		60.43^{a}	37.60^{b}	17.60		1.44^{d}	1.35	10.02^{cd}		0.37^{e}	0.55^{d}	0.68^{e}
T_{16}		58.31^{a}	61.20^{a}	26.38		1.85^{d}	0.81	5.31^{d}		0.36^{e}	0.54^{d}	0.66^{f}
C												

T_1- Pulp + MD (2%) + LDPE bags +AT T_2- Pulp + MD (2%) + LDPE bags +RT T_3 - Pulp + MD (2%) + glass jars +AT **0**
T_4- Pulp + MD (2%) + glass jars +RT T_5- Pulp + MD (3%) + LDPE bags +AT T_6- Pulp + MD (3%) + LDPE bags+RT
T_7- Pulp + MD (3%) + glass jars +AT T_8- Pulp + MD (3%) + glass jars +RT T_9 - Pulp + MD (4%) + LDPE bags+AT
T_{10}- Pulp + MD (4%) + LDPE bags +RT T_{11}- Pulp + MD (4%) + glass jars +AT T_{12}- Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT T_{14}- Pulp + MD (5%) + LDPE bags +RT T_{15}- Pulp + MD (5%) + glass jars +AT
T_{16}- Pulp + MD (5%) + glass jars +RT
MAS: Months after storage * - Unmarketable NS- Non-significant

4.4.2.14. Microbial load (bacteria, fungi and yeast)

In freeze dried avocado fruit powder, microbial population was nil up to one month of storage and after two and three months of storage, lower microbial count was noticed in samples with higher concentration of maltodextrin. In all the samples, yeast population was not observed during the storage. In T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) and T_8 (fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in refrigerated temperature), fungal population was absent after two months of storage and had lowest fungal population (0.33×10^4 cfu/g) after three months of storage. Lowest bacterial population was seen in T_{10} (fruit pulp pre-treated with 4 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) after 2 (1.17×10^5 cfu/g) and 3 (1.67×10^5 cfu/g) months of storage (Table 23).

4.4.2.15. Organoleptic evaluation

During the three months storage period of freeze dried avocado powder, organoleptic qualities decreased in all the treatments which indicated the decline of consumer acceptability (Table 24a and 24b). It was observed that organoleptic attributes such as appearance, colour and texture obtained comparatively higher scores than those for odour, flavour, taste and after taste. At the end of storage, samples became unacceptable for attributes such as odour, flavour, taste and after taste wherein overall acceptability was recorded lower than 5.0 hedonic point. Higher values of Kendall's coefficient of concordance (W) for appearance, colour and texture indicated the stronger association of judgment regarding the acceptance of samples. It was observed that T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) had highest total scores of 64.6, 61.9, 56 and 43.4 before storage as well as one, two and three months after storage, respectively.

4.4.2.16. Cost of production

Cost of production for the 1kg freeze dried avocado fruit powder from ripe avocado fruits of Rs.200/kg, pre-treated with food additives and packaged in LDPE laminated aluminium pouches and glass jars was observed as Rs.1224.86 (Table 25).

Considering the higher bulk density (T_{14}: 0.26 and T_{16}: 0.45 g/cm^3), solubility (T_{14}: 62.5 and T_{16}: 63.33 %), colour value L (T_{14}:75.98 and T_{16}: 76.16), TSS (T_{14}: 4.13 and T_{16}: 7.70 ^{o}brix), vitamin C (T_{14}: 70.00 mg/100g), total carbohydrates (T_{14}: 35.20 and T_{16}: 34.80 g/100g), total fat (T_{14}: 60.43 and T_{16}: 61.20 %), organoleptic scores and.

Table 23. Effect of food additives on microbial population of avocado fruit powder

Treatments	Initial			1MAS			2MAS			3MAS		
	Fungi	Yeast	Bacteria	Fungi	Yeast	Bacteria	Fungi	Yeast	Bacteria	Fungi	Yeast	Bacteria
T_2	0	0	0	0.00	0.00	0.00	2.67	0.00	9.83	3.33^{bc}	0.00	14.50
T_4				0.00	0.00	0.00	0.67	0.00	3.33	2.33^{bc}	0.00	6.33
T_6				0.00	0.00	0.00	4.67	0.00	2.00	8.67^{a}	0.00	7.83
T_8				0.00	0.00	0.00	0.00	0.00	4.50	0.33^{c}	0.00	8.00
T_{10}				0.00	0.00	0.00	1.00	0.00	1.17	1.67^{bc}	0.00	1.67
T_{12}				0.00	0.00	0.00	2.67	0.00	3.30	5.00^{ab}	0.00	6.50
T_{14}				0.00	0.00	0.00	0.00	0.00	3.83	0.33^{c}	0.00	6.67
T_{16}				0.00	0.00	0.00	2.67	0.00	3.00	3.67^{bc}	0.00	6.00
CD (0.05)				**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**4.58**	**NS**	**NS**

T_1- Pulp + MD (2%) + LDPE bags +AT T_2- Pulp + MD (2%) + LDPE bags +RT T_3 - Pulp + MD (2%) + glass jars +AT
T_4- Pulp + MD (2%) + glass jars +RT T_5- Pulp + MD (3%) + LDPE bags +AT T_6- Pulp + MD (3%) + LDPE bags+RT
T_7- Pulp + MD (3%) + glass jars +AT T_8- Pulp + MD (3%) + glass jars +RT T_9 - Pulp + MD (4%) + LDPE bags+AT
T_{10}- Pulp + MD (4%) + LDPE bags +RT T_{11}- Pulp + MD (4%) + glass jars +AT T_{12}- Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT T_{14}- Pulp + MD (5%) + LDPE bags +RT T_{15}- Pulp + MD (5%) + glass jars +AT
T_{16}- Pulp + MD (5%) + glass jars +RT

MAS: Months after storage * - Unmarketable NS- Non-significant

Table 24a. Effect of food additives on organoleptic scores of avocado fruit powder before and 1 month after storage

Treatments	Initial									IMAS								
	A	**C**	**TE**	**O**	**F**	**TA**	**AT**	**OA**	**TS**	**A**	**C**	**TE**	**O**	**F**	**TA**	**AT**	**OA**	**TS**
T_2	7.9	7.8	8	8	7.5	7.4	7.1	7.2	**60.9**	7.1	6.9	6.7	7.1	6.3	5.9	5.4	6.1	**51.5**
T_4	8.3	8.3	8.1	8.2	7.6	7.5	7.3	7.3	**62.6**	8.3	8.2	7.8	6.8	6.5	5.8	6.1	6.5	**56.0**
T_6	8.7	8.5	8.4	8.1	7.3	7.2	7.2	7.2	**62.6**	8.6	8.5	7.8	6.4	6.2	6.3	6	6.6	**56.4**
T_8	8.1	8.1	8.1	8.1	7.8	7.2	7.0	7.3	**61.7**	7.6	7.4	7.3	6.6	6.4	6.6	6.4	6.9	**55.2**
T_{10}	8.2	8.2	8.2	8.1	8.0	7.3	7.4	7.6	**63**	7.7	7.7	7.7	7.2	6.8	6.6	6.4	7.5	**57.6**
T_{12}	8.2	8.3	8.3	8.0	7.9	7.7	7.3	7.5	**63.2**	8.2	8.1	7.6	7.2	7.2	7.0	6.6	7.5	**59.4**
T_{14}	8.3	8.3	8.3	8.0	7.9	7.5	7.4	7.8	**63.5**	8.3	8.1	8.1	6.7	6.7	7.0	7	7.8	**59.7**
T_{16}	8.6	8.6	8.3	8.1	8.0	7.7	7.6	7.7	**64.6**	8.6	8.4	7.8	7.7	7.5	7.1	7.1	7.7	**61.9**
Kendall's W	**0.13**	**0.07**	**0.03**	**0.01**	**0.06**	**0.03**	**0.04**	**0.05**		**0.34**	**0.32**	**0.14**	**0.16**	**0.16**	**0.2**	**0.19**	**0.13**	

T_1- Pulp + MD (2%) +LDPE bags +AT
T_2- Pulp + MD (2%) +LDPE bags +RT
T_3 - Pulp + MD (2%) +glass jars +AT
T_4- Pulp + MD (2%) +glass jars +RT
T_5- Pulp + MD (3%) +LDPE bags +AT
T_6- Pulp + MD (3%) +LDPE bags +RT
T_7- Pulp + MD (3%) +glass jars +AT
T_8- Pulp + MD (3%) +glass jars +RT
T_9 - Pulp + MD (4%) + LDPE bags +AT
T_{10}- Pulp + MD (4%) + LDPE bags +RT
T_{11}- Pulp + MD (4%) + glass jars +AT
T_{12}- Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT
T_{14}- Pulp + MD (5%) + LDPE bags +RT
T_{15}- Pulp + MD (5%) + glass jars +AT
T_{16}- Pulp + MD (5%) + glass jars +RT

MAS: Months after storage
* - Unmarketable
NS- Non-significant
A: Appearance C: Colour
Te: Texture F: Flavour
Ta: Taste
At: After taste O: Odour
Oa: Overall acceptability
Ts: Total score

Table 24b. Effect of food additives on organoleptic scores of avocado fruit powder 2 and 3 months after storage

Treatments	2MAS									3MAS								
	A	**C**	**TE**	**O**	**F**	**TA**	**AT**	**OA**	**TS**	**A**	**C**	**TE**	**O**	**F**	**TA**	**AT**	**OA**	**TS**
T_2	6.7	6.4	6.4	6.4	5.8	5.0	5.1	5.6	**47.4**	5.8	5.4	5.3	3.6	4.5	2.6	3.3	3.6	**34.1**
T_4	6.9	7.5	6.9	6.3	6.3	5.6	5.5	5.8	**50.8**	5.5	6.0	5.3	4.6	4.2	3.0	2.6	4.9	**36.1**
T_6	7.8	7.2	7.5	5.9	5.9	5.6	5.3	5.7	**50.9**	6.0	5.8	5.4	5.1	4.5	3.0	3.5	5.2	**38.5**
T_8	7.3	7.1	7.0	6.2	6.1	5.6	5.6	6.0	**50.9**	6.3	6.2	5.9	4.5	4.7	2.7	3.7	4.6	**38.6**
T_{10}	7.4	7.2	7.2	6.6	6.3	5.8	5.7	6.4	**52.6**	6.5	5.6	6.1	4.5	4.8	3.3	4.2	4.4	**39.4**
T_{12}	7.5	7.4	7.6	6.6	6.7	6.2	5.9	6.1	**54.0**	5.8	6.2	6.6	5.6	5.1	3.6	3.7	5.1	**41.7**
T_{14}	7.7	7.5	7.8	6.4	6.6	6.1	6.0	6.7	**54.8**	6.0	6.4	6.6	5.4	4.8	3.5	3.6	4.9	**41.2**
T_{16}	7.8	7.7	7.8	6.8	7.1	6.2	5.9	6.7	**56.0**	6.4	6.3	6.8	5.6	5.7	4.1	3.3	5.2	**43.4**
Kendall's W	**0.11**	**0.09**	**0.11**	**0.05**	**0.09**	**0.07**	**0.06**	**0.17**		**0.53**	**0.08**	**0.23**	**0.17**	**0.10**	**0.10**	**0.06**	**0.15**	

T_1- Pulp + MD (2%) +LDPE bags +AT T_2- Pulp + MD (2%) +LDPE bags +RT T_3 - Pulp + MD (2%) +glass jars +AT
T_4- Pulp + MD (2%) +glass jars +RT T_5- Pulp + MD (3%) +LDPE bags +AT T_6- Pulp + MD (3%) +LDPE bags +RT
T_7- Pulp + MD (3%) +glass jars +AT T_8- Pulp + MD (3%) +glass jars +RT T_9 - Pulp + MD (4%) + LDPE bags +AT
T_{10}- Pulp + MD (4%) + LDPE bags +RT T_{11}- Pulp + MD (4%) + glass jars +AT T_{12}- Pulp + MD (4%) + glass jars +RT
T_{13}- Pulp + MD (5%) + LDPE bags +AT T_{14}- Pulp + MD (5%) + LDPE bags +RT T_{15}- Pulp + MD (5%) + glass jars +AT
T_{16}- Pulp + MD (5%) + glass jars +RT

MAS: Months after storage * - Unmarketable NS- Non-significant
A: Appearance C: Colour Te: Texture F: Flavour Ta: Taste
At: After taste O: Odour Oa: Overall acceptability Ts: Total score

lower hygroscopicity (T_{16}: 0.25 and T_{14}: 1.31 %), a* value (T_{14}:14.21 and T_{16}: 14.18), b* value (T_{14}: 25.30 and T_{16}:86.46), peroxide value (T_{14}: 1.44 and T_{16}: 5.31 meq./kg), water activity (T_{16}: 0.36 to 0.66) and microbial population, freeze dried avocado fruit powder with 5% maltodextrin along with additives like ascorbic acid (1%), tricalcium phosphate (0.15 %), EDTA (0.05 %) and potassium sorbate (0.05 %) packed in both aluminium laminated LDPE pouches (T_{14}) and glass jars (T_{16}) stored under refrigerated conditions were observed as the ideal pre-treatments for preservation

Table 25. Cost of production for 1kg freeze dried avocado fruit powder

Avocado powder	Rate	Quantity	Cost (Rs.)
Avocado fruits	Rs.200/Kg	2.5kg	500.00
Maltodextrin (5 %)	Rs.150/500g	50g	15.00
Ascorbic acid (1 %)	Rs.300/500g	10g	6.00
Tricalcium phosphate (0.15 %)	Rs.345/500g	1.5g	1.03
EDTA (0.05 %)	Rs.300/500g	0.5g	0.03
Potassium sorbate (0.05 %)	Rs.800/500g	0.5g	0.80
Freeze drying (3kwh)	Rs.5/unit	36 hr	540.00
LDPE laminated aluminium pouches	Rs.6/pack	2 packs	12.00
Glass jars	Rs.10/bottle	4 bottles	40.00
Labour charge	Rs.600/8hr	2 hr	40.00
Total			**Rs.1224.86/kg**

4.4.3. Preparation of instant fruit shake

Avocado fruit powder shake was standardised with skimmed milk powder, sucrose and water in the proportion of 1:2:1:2 with better sensorial acceptability. The prepared instant fruit shake had appealing appearance, light yellowish colour, unique blend of taste and flavour of avocado fruit powder and skimmed milk powder, with slight after taste of fruit powder (Plate 15).

Plate 15. Instant avocado fruit shake

DISCUSSION

5. DISCUSSION

Avocado (*Persea americana* Mill), is a subtropical, evergreen fruit tree belonging to the family Lauraceae. Krumreich *et al*. (2018) reported that the bioactive compounds in avocado pulp of Breda variety such as lipid, protein, TSS, fiber, ash, acidity, phenols and antioxidant activity were in the range of 15.8 %, 1.7 %, 8.1 $^{\circ}$Brix, 1.6 %, 0.6 %, 0.5 %, 97.3 mg gallic acid kg^{-1} and 79 % inhibition, respectively. Avocado fat composition is similar to olive oil, rich in mono-unsaturated fatty acids which reduces the low density lipoproteins in blood, are responsible for its therapeutic value (Gosh, 2000).

The unique climacteric nature of the fruit which starts ripening immediately after harvest, leads to faster spoilage and decay. Avocado based products have a very short shelf-life due to the deteriorative effects of various enzymes such as polyphenol oxidase. The major concerns regarding the processing and preservation of avocado includes maintaining the optimum ripeness and reducing discolouration by retarding the enzymatic browning due to polyphenoloxidase and overcoming off-flavour development and loss of texture (Owusu, 2011). One of the commonly used preservation methods to counteract this problem is the use of additives such as antioxidants (ascorbic acid, citric acid), preservatives (sodium benzoate and potassium sorbate), *etc* (Cortes-Rodríguez *et al*., 2019). New technologies like freeze drying is an ideal preservation method by lowering moisture content and water activity thereby, curbs the enzymatic activity and microbial growth, with the combined action of freezing and vacuuming (Owusu, 2011).

Due to the limited number of processed products, avocado fruits are consumed in the fresh form world over. As it is a seasonal fruit crop, lack of appropriate processing and storage conditions results in huge losses. Conventional avocado processing have many detrimental effects and there is scarcity of studies with new technologies regarding the processing and preservation of avocado. Thus, the present investigation was taken up to characterise avocado accessions and explore different preservation techniques and its suitability in the post harvest life of avocado.

The major observations obtained in the study titled “Post harvest characterisation and management of avocado (*Persea americana* Mill.)” are discussed in this chapter in the following sections.

5.1. Characterisation of avocado accessions

5.2. Effect of shrink packaging and storage temperature on quality and shelf life of avocado

5.3. Effect of food additives on quality of frozen avocado during storage

5.4. Effect of food additives on quality of avocado pulp and avocado fruit powder

5.1. Characterisation of avocado accessions

5.1.1. Horticultural traits

Among 27 accessions collected from different parts of Kerala, fruit shape varied from clavate to spheroid with highest and lowest fruit length, fruit diameter and fruit weight were observed in accession 13 and 14, respectively. Most of the accessions maintained high uniformity in fruit size with inflated base shape and rounded apex shape with central apex and pedicel position. In most of the accessions, fruit surface were devoid of ridges with strong glossiness and intermediate smooth skin surface in fruits collected from Wayanad while medium glossiness with smooth skin surface were observed in most of the fruits collected from Idukki. In accessions collected from Wayanad, fruits skin colour turned to purple on ripening while greenish yellow colour was noticed in ripened fruits of most of the accessions collected from Idukki. Fruit skin thickness was higher in fruits collected from Idukki than Wayanad, which were brittle with slight adherence to the flesh. Colour of the flesh next to the skin was light green and that next to the seed was light yellow. Buttery textured low fibrous flesh with neither sweet nor bitter in taste were observed in most of the accessions. General taste of flesh was fair to good while accessions such as 16, 19 and 25 had excellent taste of flesh. Discoloration was not observed in most of the accessions even four hours after cut opened the fruit except in accession 5 with grey discoloration and accession 3, 14, 22 and 26 with brown discoloration.

Avocado fruits weighing 98.8, 309.8 and 312.5 g with peel having very thin, thick and medium thickness and membrane, corky and leathery texture were reported in Mexican, Guatemalan and Antillean races, respectively (Dorantes *et al*., 2004). Manuwa and Muhammad (2010) reported that the size of fruit decreased with an increase in moisture content. Avocado fruit could be pear-shaped, egg-shaped or spherical, with 7-20 cm long and 100-1000 g weight depending on the variety (Maitera *et al*., 2014). Duarte *et al*. (2016) reported that Antillean race, originated in the lowland

regions of South America and Central America, had large fruits of pyriform shape. Guatemalan race, originating from the highlands of Central America, had round fruit and Mexican race, in the highlands of Mexico and the Andes had small fruit. The variety Quintal, hybrid of Antillean and Guatemalan races had large pyriform fruits with 500-800 g weight, while Hass variety had an average size of 180 to 300 g (Duarte *et al*., 2016).

Fruits of Mexican race had thin smooth skin while Guatemalan races had thick and often warty fruit skin and in West Indian races fruit skin was smooth, leathery and glossy textured. Hass variety had fruits with pebby green skin forming purplish black when ripened with creamy, pale green flesh. Fuerte variety had smooth green fruits of high quality with creamy, pale green flesh which was easily peelable (Tripathi and Sanker, 2014).

The fruits of variety Quintal had smooth greenish coloured skin and yellow flesh with less adherence to skin (Duarte *et al*., 2016). Nnaji and Okereke (2016) reported that the pulp of mature avocado fruit had sweet pleasant taste.

Augustine (2020) characterised avocado fruits collected from Ambalavayal in Wayanad, in which avocado fruits appeared in narrowly obovate, clavate, pyriform, ellipsoid and spheroid shapes with fruit weight in the range of 152.40 g to 434.20 g, fruit length varied from 7.68 to 14.66 cm, fruit diameter from 5.30 cm to 8.42 cm and skin thickness of 1 mm. Depressed base shape and flattened apex shape without ridges, intermediate glossiness and smooth skin surface were most commonly seen. Purple or light green coloured skin with light green and light yellow colour in flesh next to skin and next to seed, respectively were reported. Buttery textured flesh with low degree of discoloration even four hours after cut opened the fruit was commonly observed among the 25 accessions collected from Wayanad.

5.1.2. Biochemical characterisation

5.1.2.1. Total soluble solids

Among the 27 genotypes of avocado collected from Wayanad, Idukki and Thrissur districts of Kerala, total soluble solids (TSS) were observed in the range of 4.27 ºBrix in Acc. 20 to 11.33 ºBrix in Acc. 13.

Astudillo-Ordonez and Rodrigez (2018) reported a range of 5.07 and 7.26 ºBrix of total soluble solids in avocado fruits before storage. Kruger *et al*. (2019) observed

an increase in TSS content of avocado fruits in the orchard from 7.70 to 10.50 ºBrix in early season varieties while it varied from 8 to 11 ºBrix in late season varieties. The study revealed that the lowest TSS value indicated the end of maturation phase and the starting of on-tree ripening. Augustine (2020) reported that the TSS content in 25 accessions of avocado fruits collected from Ambalavayal in Wayanad varied in the range of 6.30 to 10.60 ºBrix. Kassim and Workneh, (2020) reported total soluble solids of 2.90 ºBrix in avocado fruits before storage.

5.1.2.2. Titratable acidity

Titratable acidity varied significantly among the accessions collected from Kerala which ranged from 0.28 to 2.84 % in Acc 27 and Acc 12, respectively.

Ahmed *et al*. (2010) reported mild decrease of titratable acidity during ripening in avocado cv. Fuerte. As the ripening advanced, total acid content in avocado (tartaric, malic, citric and ascorbic acids) decreased with major decrease in malic acid which resulted in the decrease of titrable acidity (Defilippi *et al*., 2015). Among the 25 accessions of avocado fruits collected from Ambalavayal in Wayanad titratable acidity was observed in the range of 0.64 to 1.28 % (Augustine, 2020). Kassim and Workneh, (2020) reported titratable acidity of 1.7 to 1.9 $mg.mL^{-1}$ in fresh avocado fruits without any treatments or storage.

5.1.2.3. Vitamin C

Vitamin C content in ripe avocado fruit varied from 5.33 mg/100g in Acc. 18 and Acc. 20, collected from Idukki, to 20.00 mg/100g in Acc. 11, from Wayanad. Lower vitamin C was observed in accessions collected from Wayanad and highest in that from Idukki.

According to Dreher and Davenport (2013) edible portion of Hass avocado contained 8.80 mg ascorbic acid/100 g FW. Tripathi and Sanker (2014) mentioned chemical composition of avocado fruit per 100g of edible portion in which ascorbic acid content was about 16.00 mg. Pedreschi *et al*. (2019) reported an average content of vitamin C of 0.41 mg gDM^{-1} of mesocarp tissue and observed a decrease with fruit development.

5.1.2.4. Total carbohydrates

Total carbohydrates in ripe avocado fruits were comparatively low and in the range of 0.40 g/100g in Acc. 7 to 3.81 g/100g in Acc. 25.

Dorantes *et al*. (2004) and Soares and Ito (2015) mentioned that the biochemical composition of avocado pulp in which total carbohydrate content was in the range of 3.70 to 5.80 g/100g and 0.80 to 4.80 %, respectively. Nnaji and Okereke, (2016)

reported total carbohydrates in the range of 4.90 – 8.64 % in the pulp of various avocado varieties such as Brogdon, Russel and Choquette.

5.1.2.5. Total protein

In the present study, fruits collected from Wayanad (0.50 to 1.59 g/100g) had lower protein content than that from Idukki (8.33 to 11.93 g/100g). The total protein was observed in the range of 0.50 g/100g in Acc. 9 to 11.93 g/100g in Acc. 15.

According to Soares and Ito (2000) and FAO (2004), the protein content in avocado fruits was in the range of 1.00 to 3.00 % and 1.37 to 1.81 g/100g, respectively. Nair and Chandran (2018) reported total protein content of 1.32, 1.27, 1.23 and 1.15 g per 100g of pulp in avocado cultivars available in Kerala such as Pollock, Kallar Round, Purple Hybrid and Fuerte, respectively.

5.1.2.6. Total phenols

Total phenols in the fruits of 27 accessions collected from Kerala was in the range of 33.33 mg/100g in Acc. 22 to 102.83 mg/100g in Acc. 12, which were from Idukki and Wayanad districts, respectively. Total phenolic content was higher in fruits collected from Wayanad.

Hurtado-Fernández *et al*. (2015) reported that the concentration of phenolic acids generally decreased as the fruit ripened. Cenobio-Galindo *et al*. (2019) observed that the total phenolic content of 238.14 to 247.33 mg GAE/100g in nanoemulsion coated Hass avocado, during the initial period of storage. Phenolic compounds were stimulated under stressed conditions which contributeed to the antioxidant activity of avocado under storage conditions (Cenobio-Galindo *et al*., 2019).

5.1.2.7. Total flavonoid

Total flavonoid content varied from 24.80 mg/100g in Acc. 9 to 66.67 mg/100g in Acc. 25, which were the accessions collected from Wayanad and Idukki, respectively.

Alonso *et al*. (2017) reported flavonoid content of about 0.29 to 0.75 mg QE eq./g of fruit sample in dry weight basis. Total flavonoid content in avocado edible oil extract was observed as 0.19 ±0.02 mg RE/g (Xaun *et al*., 2018). Cenobio-Galindo *et al*. (2019) observed 36.04 to 37.07 mg QE/100g in nanoemulsion coated Hass avocado during the initial period of storage. Cenobio-Galindo *et al*. (2019) elucidated that the important flavonoids in avocado were catechin and epicatechin, having potential of antioxidant activity and it increased in fruits under stressed situation. The increase in

total flavonoid content in avocado mesocarp during storage of 60 days varied from 32.00 to 48.00 mg QE/100g among different treatments with nanoemulsion.

5.1.2.8. Total fat

Total fat extracted from the fruits of 27 accessions collected from Kerala, varied significantly from 0.79 to 10.02 % in Acc.4 and Acc.7, respectively.

The oil content of avocado gradually increase with ripening which can be considered as an important index of avocado fruit ripening. Local climatic conditions, altitude and irrigation are important factors that have significant effect in the fat content of avocado fruits. Fruits of West Indian race and West Indian–Guatemalan hybrids had lower oil content, less taste, reduced shelf life and limited consumer acceptance than Guatemalan/Mexican types (Litz *et al*., 2005). Mostert *et al*. (2007) reported that ripe fruits of avocado had higher oil yield than unripe fruits. Ariza *et al*. (2011) reported 15.80 % fat content in Hass avocado fruits grown in Mexico. Castaneda-Saucedo *et al*. (2014) observed 4 % less total fat in rainfed crops than irrigated crops and also 13 % lower oleic acid in rainfed crops. It can be related with the proper utilization of irrigation water, resulting in the increase of net assimilation of CO_2 and accumulation of photoassimilates. The fat content in West Indian race was 2.59 to 11.80 % as reported by Teng *et al.* (2016). Augustine (2020) reported that fat content in avocado fruits collected from Ambalavayal was in the range of 2.63 to 6.78 %.

5.1.2.9. Oleic acid

In the present study, oleic acid content in avocado fruit accessions collected from Wayanad, Idukki and Thrissur districts of Kerala ranged from 13.49 to 86.86 g/100g in Acc. 6 and Acc. 10, respectively.

Avocado oil contains monounsaturated fatty acids mainly oleic acid which makes its biochemical composition similar to olive oil. Tovar (2003) and Sanchez (2012) reported 64.87 and 44.4 % of oleic acid in fresh avocado pulp, respectively. Avocado pulp with oleic acid content of 41.91 to 43.37 % was obtained in 250 mL hexane for 2 hours extraction (Gatbonton *et al.*, 2013).

5.1.2.10. Calcium

Calcium content in fresh avocado fruits characterised in the present study varied among genotypes from 4.90 mg/100g in Acc. 16 to 13.46 mg/100g in Acc. 25.

Tripathi and Sankar (2014) reported 10.00 mg of calcium content in avocado fruit samples and Nair and Chandran (2018) reported about 9.00, 9.20, 9.50 and 9.80 mg per 100g in avocado cultivars Pollock, Fuerte, Kallar Round and Purple Hybrid,

respectively. Maitera *et al*. (2014) mentioned about the nutritional composition of avocado pulp in which calcium content was 12.00 mg/100g.

5.1.2.11. Potassium

Potassium content in fresh avocado fruits characterised in the present study ranged from 122.27 mg/100g in Acc.16 to 460.00 mg/100g in Acc.10. Higher potassium content was observed in fruits collected from Wayanad than that from Idukki.

Maitera *et al*. (2014) mentioned that avocado contained 35 % more potassium than banana. Durante *et al*. (2016) reported about 339.00 mg/100g of potassium content in avocado. Nair and Chandran (2018) reported about 300, 400, 500 and 525 mg/100g of potassium in different cultivars such as Pollock, Kallar Round, Purple Hybrid and Fuerte, respectively.

5.1.2.12. Iron

In the present study, iron content obtained in fresh and mature avocado fruits varied from 0.04 in Acc. 17 to 0.44 mg/100g in Acc. 26.

Maitera *et al*. (2014) observed 0.069 mg/100g of iron content in avocado fruit samples. Tripathi and Sankar, (2014) reported about 0.60 mg/100g, Nair and Chandran (2018) obtained 0.40-0.60 mg/100g in different cultivars of avocado cultivated in Kerala.

5.1.2.13. Total ash

In the present study, total ash content of avocado fruits varied significantly from 0.27 % to 1.79 % in Acc. 8 and Acc. 3, respectively which represented the total mineral composition in the fruits.

FAO (1989) and USDA (2009) reported 0.4-1.68 % and 0.91 % of ash content in fresh avocado fruits, respectively. Orhevba and Jinadu (2011) reported about 1.52 % ash content in variety Fuerte. Castaneda-Saucedo *et al*. (2014) reported about 9.40 and 10.33 g/100g of ashes in avocado fruit pulp grown under rainfed and irrigated conditions, respectively. Tripathi and Sankar (2014) reported total ash content of avocado fruits as 0.58-0.89 g $100g^{-1}$. Nnaji and Okereke (2016) reported 1.00 to 1.07 % ash content in the pulp of various avocado varieties such as Brogdon, Russel and Choquette.

5.1.2.14. Crude fibre

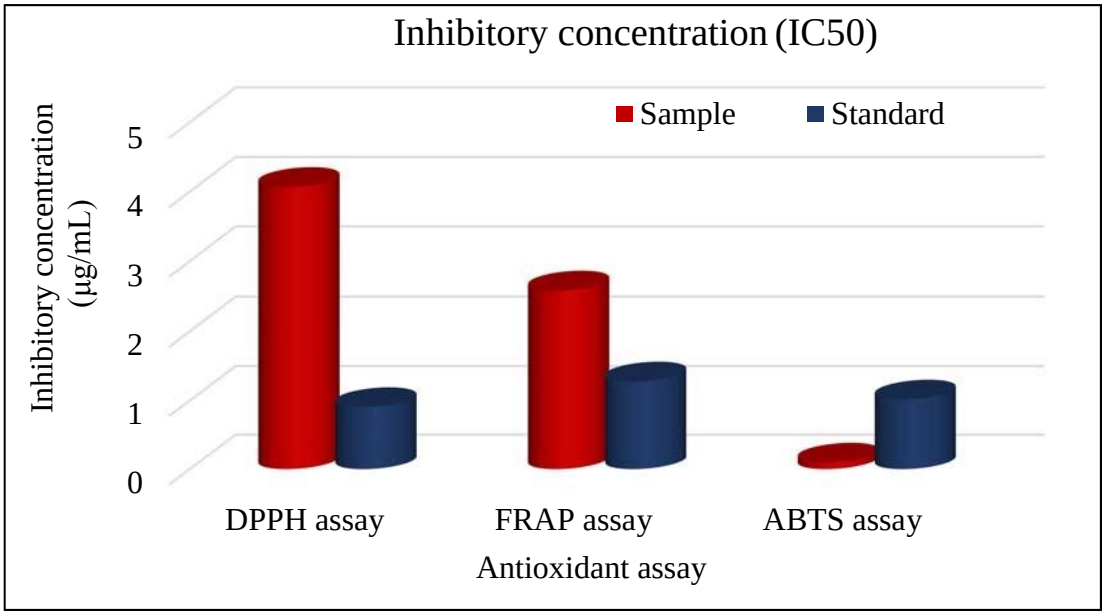
Avocado fruits of 27 accessions collected from Kerala had crude fibre content in the range of 2.24 to 7.61 % in Acc. 12 and Acc. 6, respectively.

Avocado has higher fibre content among fruits with 75 % insoluble and 25 % soluble fibre. Fibre content in fruit sample have regulatory effect in the appetite as well as in the bowel movement. Soares and Ito (2015) reported crude fibre content of avocado fruits with 1.40 to 3.00 %. USDA (2009) suggested crude fibre content in avocado fruits as 6.70 g $100g^{-1}$ and Orhevba and Jinadu (2011) reported 6.90 g $100g^{-1}$ in variety Fuerte. Castaneda-Saucedo *et al*. (2014) and Maitera *et al*. (2014) reported that crude fibre content in avocado fruit was 6.10 to 6.23 g/100g and 4.03 %, respectively. Nnaji and Okereke (2016) reported that crude fibre content in fresh avocado fruit ranged from 4.15 to 4.80 %.

5.1.2.15. Antioxidant activity (DPPH, ABTS, FRAP)

Inhibitory concentration (IC_{50}), indicating the concentration of antioxidant in the avocado fruit capable for scavenging 50 % of the free radicals was analysed using DPPH, FRAP and ABTS assays (Fig.1). IC_{50} value observed in the DPPH, FRAP and ABTS assays of fresh avocado fruit were 4.07 µg/mL, 2.58 µg/mL and 0.10 µg/mL, respectively. Lowest IC_{50} value in ABTS assay indicated the highest antioxidant activity followed by FRAP and DPPH assays. Vieites *et al*. (2012) recorded antioxidant activity of avocado cv. 'Fuerte' in DPPH assay in the range of 17.60 to 68.70 %, with higher values for fruits at room temperature and an increasing trend was observed after respiratory peak of fruits during storage. Wang *et al*. (2012) reported higher antioxidant capacity in early harvested (January) Hass avocado fruit than late harvested (June) during storage period of 35 days. In DPPH assay, it was in the range of 37.29 to 60.29 %, 37.03 to 65.28 % and 38.04 to 69.59 % at 0, 21 and 35 days of cold storage, respectively. While in FRAP assay, it was 1.42 to 2.46, 1.46 to 2.72 and 1.44 to 2.93 µmol Fe^{2+}/g FW at 0, 21 and 35 days of cold storage, respectively.

Fig. 1. Antioxidant activity of superior accession (Acc. 25)



Archana (2019) reported DPPH radical scavenging activity in avocado cultivars collected from Ambalavayal such as Fuerte, Purple Hybrid, Kallar Round and Pollock as 40.76, 34.03, 38.98 and 42.52 µg/mL, respectively. Cenobio-Galindo *et al*. (2019) reported a corresponding change in the antioxidant activity with respect to the bioactive compounds such as phenols and flavonoids while an increase in antioxidant activity was reported with increase in storage time.

Antioxidant activity of avocado mesocarp treated with 25 % nanoemulsions assessed using DPPH and ABTS assays increased from 309.00 to 435.00 mg AAE/100g and 211.40 to 233.87 mg AAE/100g and in 50 % nanoemulsions from 242 to 439 and 112 to 310 mg AAE/100g, respectively after 30 and 60 days of storage, respectively (Cenobio-Galindo *et al*., 2019).

Chaiyasut *et al*. (2019) reported an initial antioxidant activity of 0.11-0.16 and 0.28-0.42 mg Trolox equivalent/g of avocado oil in DPPH and ABTS assays, respectively. In the study it was concluded that avocado oil stored in dry cool conditions along with protection from light in amber coloured bottles retained the quality up to 3 months without any significant changes in acid value, peroxide value, total phenolic content and antioxidant activity.

5.2. Effect of shrink packaging and storage temperature on quality and shelf life of avocado.

5.2.1. Physical and physiological parameters

5.2.1.1. Shelf life

In the present study, avocado fruits with calcium chloride pre-treatment and shrink packaging along with storage under refrigeration (4-7 ºC) and cold storage (12-13 ºC) were recorded with longest shelf life of 27 days. In the fruits kept as control, under refrigeration and cool chamber storage, shelf life increased to 20 and 22 days, respectively and in ambient condition it was only 7 days. In calcium chloride pre-treated fruits, shelf life increased to 10, 20 and 24 days under ambient, refrigeration and cool chamber, respectively (Fig.2.).

Powell (1988) reported that shelf life of avocado was extended from 3.9 to 5.5 days and 4.0 to 6.3 days after 4 and 6 weeks of cold storage (5.5 ºC), respectively by the use of multi-layered film with 35-57 % oxygen permeability. Shelf life of avocado can be extended by delayed ripening due to tissue softening, cell wall disintegration, and pigment degradation by reducing the ethylene production and respiration rate (Alvarez *et al*., 2012). The shelf life of firm avocados can be extended up to 6 weeks under Controlled Atmospheric Storage (CAS) conditions of 5–12 ºC, 95 % relative humidity (RH), 2–5 % O_2 and 3-10 % CO_2 (Munhuweyi *et al*., 2019). Low temperature is important in extending shelf life of avocados by retarding the metabolism by reducing respiration rates, ethylene evolution, softening and colour change (Kassim and Workneh, 2020).

5.2.1.2. Physiological Loss in Weight (PLW)

Physiological Loss in Weight increased during storage with the increase in respiration and metabolism, which indicated the senescence of the fruits. Lower PLW was observed in shrink packaged fruits under refrigeration (T_8) followed by those under cool chamber (T_9) during 27 days of storage (Fig.3.).

Illeperuma and Nikapitiya (2015) reported that in thinner packaging material, higher water vapour permeability contributed to lower moisture condensation inside the package but significant effect was not observed in weight gain/loss. Olivares *et al*. (2020) reported that avocado fruits in the advanced maturity stage had a higher weight loss than fruits at the early maturity stage. In "Fuerte" cultivar of avocado, fruits at the early maturity stage stored for 30 days had weight loss less than 3 % and for 50 days it

Fig. 2 Effect of shrink packaging and storage temperature on shelf life (days) of avocado

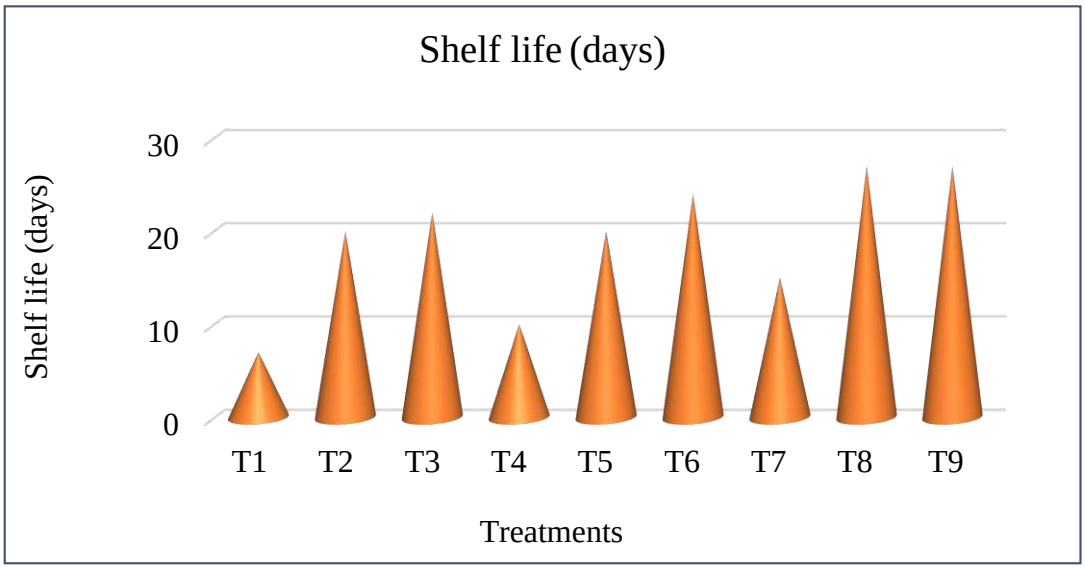
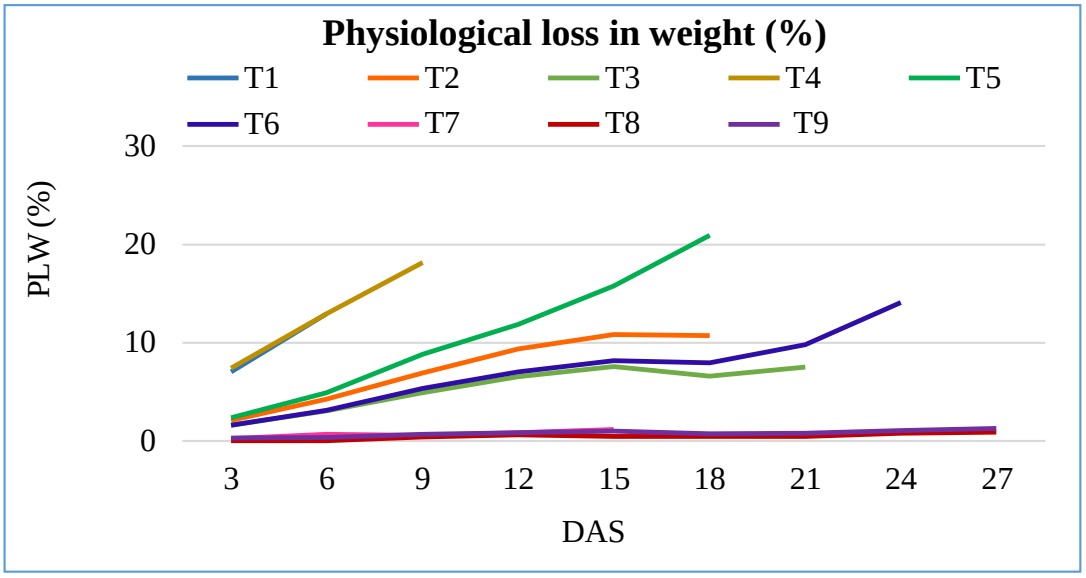


Fig. 3. Effect of shrink packaging and storage temperature on physiological loss in weight (PLW) (%) of avocado during storage



T_1- Control + ambient condition
T_2- Control + refrigeration
T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition
T_5- $CaCl_2$ + refrigeration
T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$+ shrink packaging + ambient condition
T_8- $CaCl_2$+ shrink packaging + refrigeration
T_9- $CaCl_2$+ shrink packaging + cool chamber
DAS: Days after storage

was less than 3.5 %, in room temperature and lower values close to 1 % obtained in fruits under controlled atmosphere storage. The use of plastic films and refrigeration reduced weight loss and packaged fruits of avocado under ambient condition showed higher loss of moisture which resulted in higher PLW (Olivares *et al*., 2020).

5.2.1.3. Respiration rate

The least respiration rate with decreasing trend was noticed in calcium chloride pre-treated shrink packaged fruits under refrigerated storage during 27 days of storage and climacteric peak was observed in all the treatments which were more pronounced in samples stored under cool chamber. In fast ripened fruits, climacteric peak was noticed in the early days of storage while in treatments with longer shelf life such as calcium chloride pre-treated shrink packaged fruits in cool chamber and refrigeration, climacteric peak was noticed after 15 and 18 days of storage, respectively indicated low rate of respiration (Fig.4).

The rate of respiration of avocado fruit exhibited a typical climacteric pattern during storage. Pathirana *et al*. (2013) observed the climacteric respiratory peak of control 4 days after transferring avocado fruits from 5 ºC to 20 ºC, and respiration rate decreased rapidly afterward. In the study it was mentioned that low O_2 significantly inhibited the peak value of respiration rate. Blakey *et al*. (2014) reported that Hass avocado fruits stored at 1 ºC in cold storage had significantly reduced respiration rates, ethylene evolution rates, rate of softening, water loss and fresh weight (FW) loss during 28 days of storage life.

Illeperuma and Nikapitiya (2015) reported that lower gas permeability of the thicker packaging material decreased O_2 concentration and increased CO_2 concentration in the atmosphere surrounding the avocado fruit inside the package. The O_2 concentration fell from 14.1 % to 6.3 % and 10.8 % to 3.8 % while CO_2 concentration rose from 4.7 % to 4.9 % and 3.8 % to 8.1 % during 10 days of storage in 0.050 mm and 0.075 mm thick LDPE packages, respectively.

Chen *et al*. (2017) observed a respiratory peak of 81 $mg.kg^{-1}h^{-1}$ after 6 days of storage in naturally ripened Hass avocado fruit. In Fuerte, the CO_2 production rate at harvest was similar at all the maturity stages and the respiration rate increased in fruits stored under regular air storage (Olivares *et al*., 2020).

5.2.1.4. Ethylene evolution rate

The lowest ethylene evolution rate was seen in calcium chloride pre-treated fruits under cool chamber (T_6) during 24 days of storage followed by calcium chloride pre-treated shrink packaged fruits under refrigeration (T_8) and cool chamber (T_9) with a peak value after 18 and 24 days of storage, respectively (Fig.5.).

The ethylene production rate in avocado variety "Edranol" was three to five times higher in fruits stored under regular atmosphere (RA) after 50 days of storage than that stored for 30 days, mainly due to the advanced ripening process in regular atmosphere (Jeong *et al*., 2003). Pathirana *et al*. (2013) reported that the rate of ethylene evolution of avocado fruit exhibited a typical climacteric pattern during storage which was observed 4 days after stored at 20 ºC in control. It was also mentioned that low O_2 significantly suppressed ethylene production and delayed the peak of ethylene. Avocado fruit after 6 weeks storage in CA at 5 ºC ripened significantly more slowly than 7 ºC and in air at 5 ºC (Burdon *et al*., 2017). Chen *et al*. (2017) observed that in naturally ripened Hass avocado fruit, the ethylene evolution rate attained peak value of 38 to 41 mL $kg^{-1}h^{-1}$ after 6 days of storage. Increased ethylene production was observed in "Fuerte" and "Edranol" cultivars of avocado fruits stored under regular air storage until the ready-to-eat stage (Olivares *et al*., 2020).

5.2.1.5. Texture

The firmness of the fruits decreased during storage with the progress of ripening process such as respiration and metabolism and subsequent softening of fruit. Highest retention of firmness during storage was noticed in fruits under refrigeration and cool chamber storage. The rate of decrease in texture was lower in calcium chloride pre-treated shrink packaged fruits under cool chamber (T_9) which was from 6.60 to 0.33 kg/cm^2 and in refrigeration (T_8) from 10.55 to 0.15 kg/cm^2 during 27 days of storage. Fruits stored in cool chamber (T_3 and T_6) had also maintained better firmness during storage (Fig.6).

Softening in avocado fruits is associated with the increase in hydrolytic enzymatic reactions and subsequent breakdown of cell wall (Kanellis *et al*., 1989). Partially ripened fruits obtained a firmness of about 12.5 ±0.6 kg cm^{-2} (Nikapitiya and Illeperuma, 2003), higher than the results obtained, which may be due to the faster ripening characteristic of avocado fruits. Fruits stored under RA at 5 ºC for 3.5 weeks began to soften during storage and reached the ready-to-eat stage a week later when exposed to 20 ºC (Hershkovitz *et al*., 2005). Burdon *et al*. (2017) reported that after 6

Fig.4. Effect of shrink packaging and storage temperature on respiration rate (% CO_2) of avocado during storage

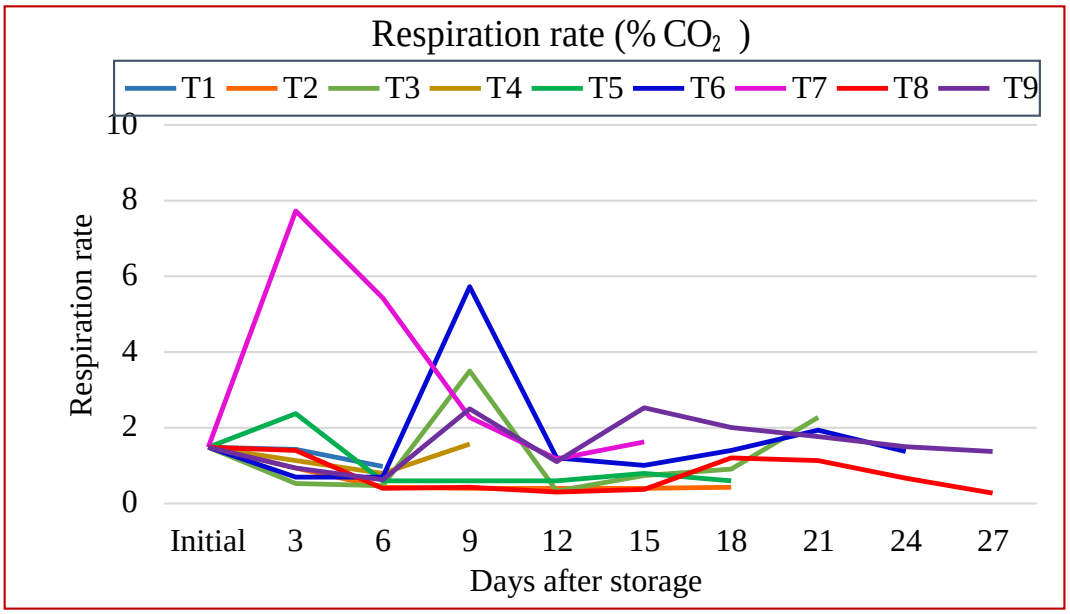
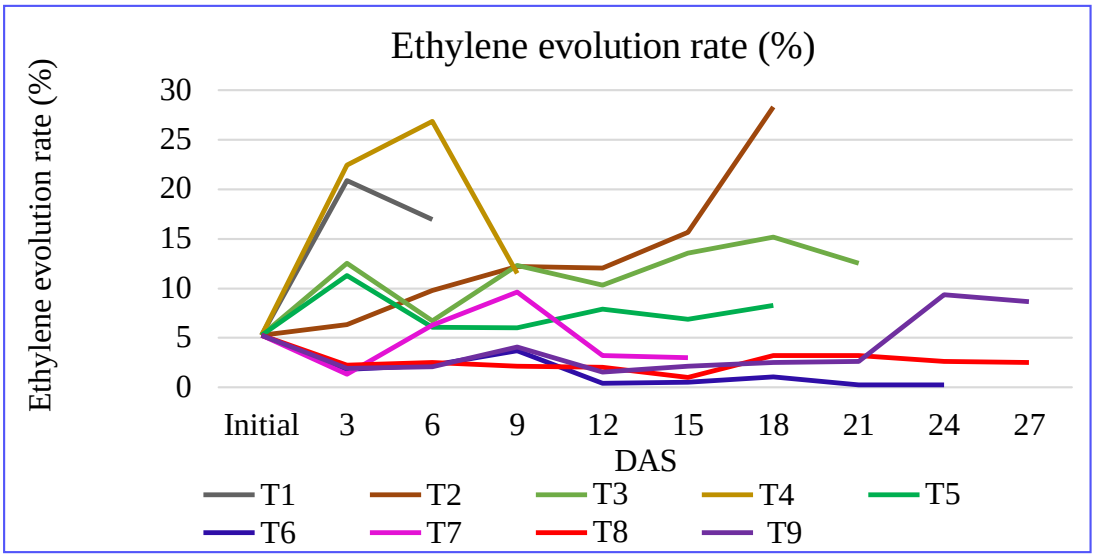


Fig. 5. Effect of shrink packaging and storage temperature on ethylene evolution rate (%) of avocado during storage



T_1- Control + ambient condition T_2- Control + refrigeration
T_3- Control + cool chamber T_4- $CaCl_2$ + ambient condition
T_5- $CaCl_2$ + refrigeration T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$+ shrink packaging + ambient condition
T_8- $CaCl_2$+ shrink packaging + refrigeration
T_9- $CaCl_2$+ shrink packaging + cool chamber DAS: Days after storage

weeks of storage, avocado fruits stored at 5 ºC and 7 ºC in CA retained the firmness with 96.1 and 87.3 N, respectively while fruits in RA at 5 ºC became soft (67.7 N). Arpaia *et al*. (2015) mentioned that in Hass avocado fruits 'near ripe' and 'eating firmness' could be determined from the firmness values close to 19 N and 4.4–6.7 N, respectively. Mazhar (2015) mentioned the importance of post harvest application of calcium in strengthening the cell wall thereby maintaining the fruit texture and firmness, and reducing the rate of ripening. It reduced the severity of bruising on avocado fruit surface. Nikapitiya and Illeperuma (2015) reported reduction in firmness of avocado slices from 12.5 to 6.3 kg cm^{-2} after 3 days and further to 5.8 kg cm^{-2} after 10 days of storage at 8 ºC when the fruits can be recognised as at table-ripe stage. "Fuerte" cultivars of avocado at all maturity stages stored under RA had a sharp decrease in flesh firmness during storage, from 230 N at harvest to 70 N and 20 N after 30 and 50 days, respectively. Fruits stored under CA maintained firmness levels independent of the storage period or maturity stage (Olivares *et al*., 2020).

Ma *et al*. (2020) reported that the textural properties of avocado flesh varied depending on different compression locations such as head, middle and tail, and compression speed and distance which may significantly determine the key indicators of avocado texture such as hardness, springiness and cohesiveness.

5.2.1.6. Decay (%)

In the present study, avocado fruits stored as control without any packaging under ambient storage (T_1) decayed completely within 9 days after storage. Control and calcium chloride pre-treated fruits under refrigeration remained without 100 per cent decay up to 21 days of storage. In control and calcium chloride pre-treated fruits in cool chamber, 100 per cent decay was observed after 24 days of storage. Calcium chloride pre-treated shrink packaged fruits under refrigeration and cool chamber storage remained without 100 percent decay up to 27 days of storage. The shrink packaged fruits retained the freshness for longer days of storage with slow rate of decay (Fig.7). Internal damage includes grey pulp (mesocarp discoloration starting at the bottom of the fruit adjacent to the seed and spreading upwards and outwards) and vascular browning (brown vascular bundle discoloration at the stem end of the fruit at advanced maturity stage under RA) and pulp spot (black or grey spots along the margins of the vascular bundles, distributed randomly throughout the fruit). Lower susceptibility to pulp spot shows the capacity to withstand low temperature at the advanced maturity stage.

The incidence of external damage was observed immediately after storage in fruit at the early maturity stage stored under RA. External damage in avocado is associated with the development of physiological disorders related to chilling injury which may result in skin browning, internal browning, vascular browning and increased pathogen susceptibility (Woolf *et al*., 2003).

Fruits stored for 6 weeks at 2 or 5 ºC had greater incidence and severity of stem end rot and brown patches than fruits stored for 4 weeks. Internal chilling injury was related to both maturity and storage duration with the late season fruit having the greatest incidence and severity of diffuse flesh discolouration (Dixon *et al*., 2004).

After 6 weeks of storage, the incidence of rots in the ripe fruit was affected by the storage environment. Lowest incidence of stem end rot (SER), body rot (BR) and vascular browning (VB) were observed in fruit stored in CA at 5 ºC. There was no increase in the diffuse flesh discoloration (DFD) incidence with storage time for samples under CA storage. There was large decrease in the quality of fruit when air storage was extended from 4 to 6 weeks compared to CA stored fruit (Burdon *et al*., 2017).

5.2.1.7. Post harvest disease incidence

During post harvest storage of avocado, fruits were mainly infected with diseases like anthracnose, fruit rot and stem end rot, which were caused by *Collectotrichum gloeosporioides*.

Avocado is susceptible to decay within a few days after ripening. Durand (1984) reported least post harvest disease symptoms in avocado fruits packed with shrink packaging material. The most common diseases are stem end rot, anthracnose and *Collectotrichum* fruit rot (Korsten *et al.,* 1988). Menge and Ploetz (2003) reported higher incidence of tissue breakdown related with stem end rot and body rot under hot and humid climate caused by *Colletotrichum gloeosporioides*, causal organism of anthracnose also. On microbial analysis among three randomly selected decayed fruits showed the presence of *Collectotrichum*, which caused stem end rot as well as anthracnose. Regardless of the month of harvest, storage at 5 ºC for 4 weeks would be suitable for keeping the optimum quality of avocado fruits (Dixon *et al*., 2004). The incidence of mold at the peduncular scar of the fruit was observed mainly in avocado stored under RA at 5 ºC after 30 days. In fruit at the advanced maturity stage, the incidence of damage increased with the storage period, reaching 80 % in avocado under

Fig.6. Effect of shrink packaging and storage temperature on texture (kg/cm²) of avocado during storage

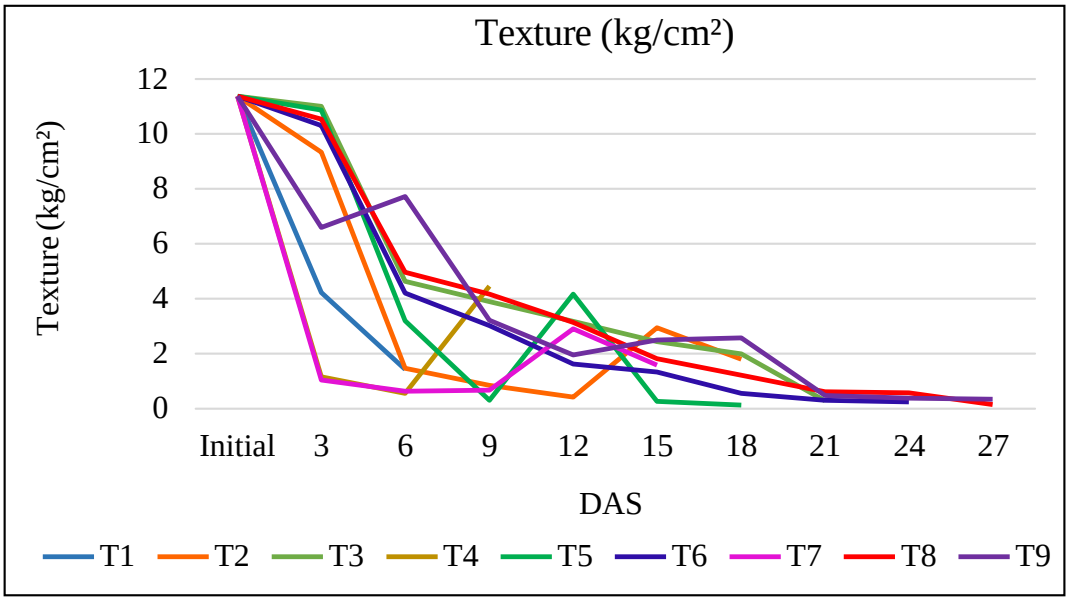
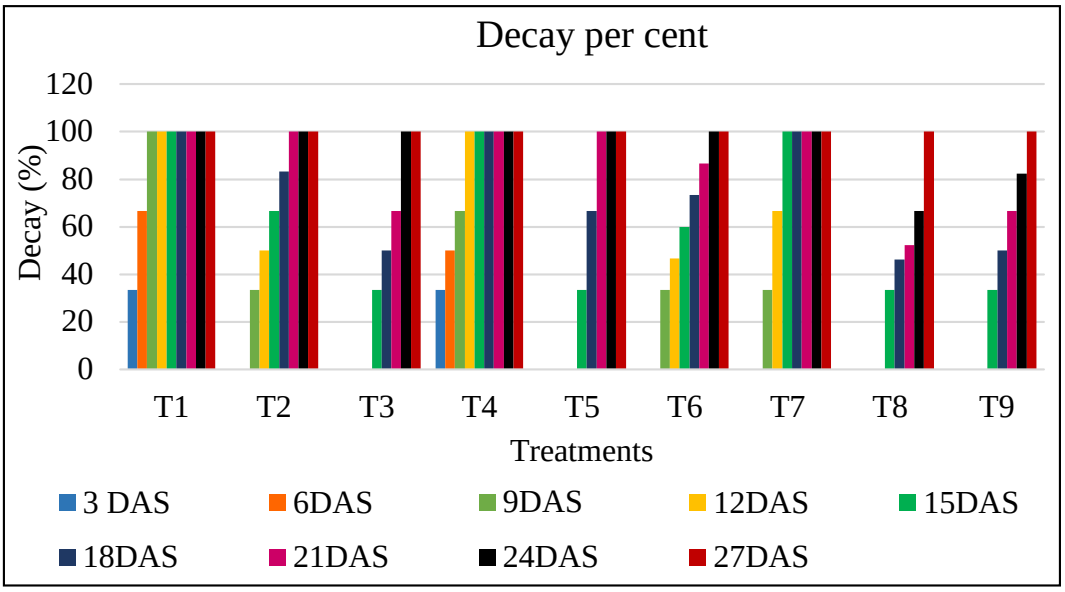


Fig. 7. Effect of shrink packaging and storage temperature on decay (%) of avocado during storage



T_1- Control + ambient condition T_2- Control + refrigeration

T_3- Control + cool chamber T_4- $CaCl_2$ + ambient condition

T_5- $CaCl_2$ + refrigeration T_6- $CaCl_2$ + cool chamber

T_7- $CaCl_2$+ shrink packaging + ambient condition

T_8- $CaCl_2$+ shrink packaging + refrigeration

T_9- $CaCl_2$+ shrink packaging + cool chamber DAS: Days after storage

RA after 50 days. Avocado fruits stored in CA at 7 ºC had lower incidence of fuzzy patches (FP) due to fungal infection and discrete patches (DP) due to physiological disorder than those stored in either CA or RA at 5 ºC (Burdon *et al*., 2017). Augustine (2020) reported anthracnose caused by *Collectotrichum gloeosporioides* and scab by *Sphaceloma perseae* in avocado fruits collected from Wayanad.

5.2.2. Biochemical parameters

5.2.2.1. Total Soluble Solids

In the study, significantly highest value of total soluble solids was observed in calcium chloride pre-treated fruits under cool chamber (T_6) throughout the storage period of three weeks. TSS in avocado fruits increased during storage which might be due to the conversion of starch into soluble sugars during ripening and osmotic effect of calcium chloride. Lowest TSS content in shrink packaged fruits under refrigeration and cool chamber indicated the slower rate of ripening (Fig. 8a).

Aguirre-Joya *et al.* (2017) observed a general increase in the TSS of avocado fruit during storage without any significant change in both refrigerated and room storage conditions. There was an increase in brix degrees (5.07 to 7.26) and pH (6.58 to 7.14) throughout the storage time until the fourth week, which decreased afterwards. In contrast, acidity dropped (9.47 to 9.24 %) with storage time (Astudillo-Ordonez and Rodrigez, 2018).

Total soluble solids increased in general throughout the storage due to the hydrolysis of carbohydrates stored within the fruits into soluble sugars, which was in faster rate at ambient conditions under higher temperature and reduced relative humidity. Lower rate of increase in TSS indicated the slower rate of senescence and ripening, and longer shelf life (Kassim and Workneh, 2020).

5.2.2.2. Titratable acidity

Titratable acidity decreased after one week of storage and increased towards the end of storage. Significantly highest values were found in shrink packaged fruits under ambient (T_7), refrigeration (T_8) and cool chamber (T_9), two weeks after storage indicated the lower rate of biochemical and metabolic reactions and subsequent ripening of the fruits (Fig. 8a).

Kassim and Workneh (2020) observed an increase in titratable acidity during all the storage conditions of ambient, refrigeration and cold storage, but it increased at a faster rate under ambient condition (1.9 to 11.5 %) than cold storage (1.7 to 7.5 %)

which extended the shelf life by 14 more days. It was also reported that fruits under packaged conditions had lower titratable acidity than those under unpackaged conditions.

5.2.2.3. Total carbohydrates

Total carbohydrates decreased during storage and higher content were observed in control under ambient condition (T_1), calcium chloride pre-treated fruits under refrigeration (T_5) and calcium chloride pre-treated, shrink packaged fruits under refrigeration (T_8) after 1, 2 and 3 weeks of storage, respectively (Fig. 8a).

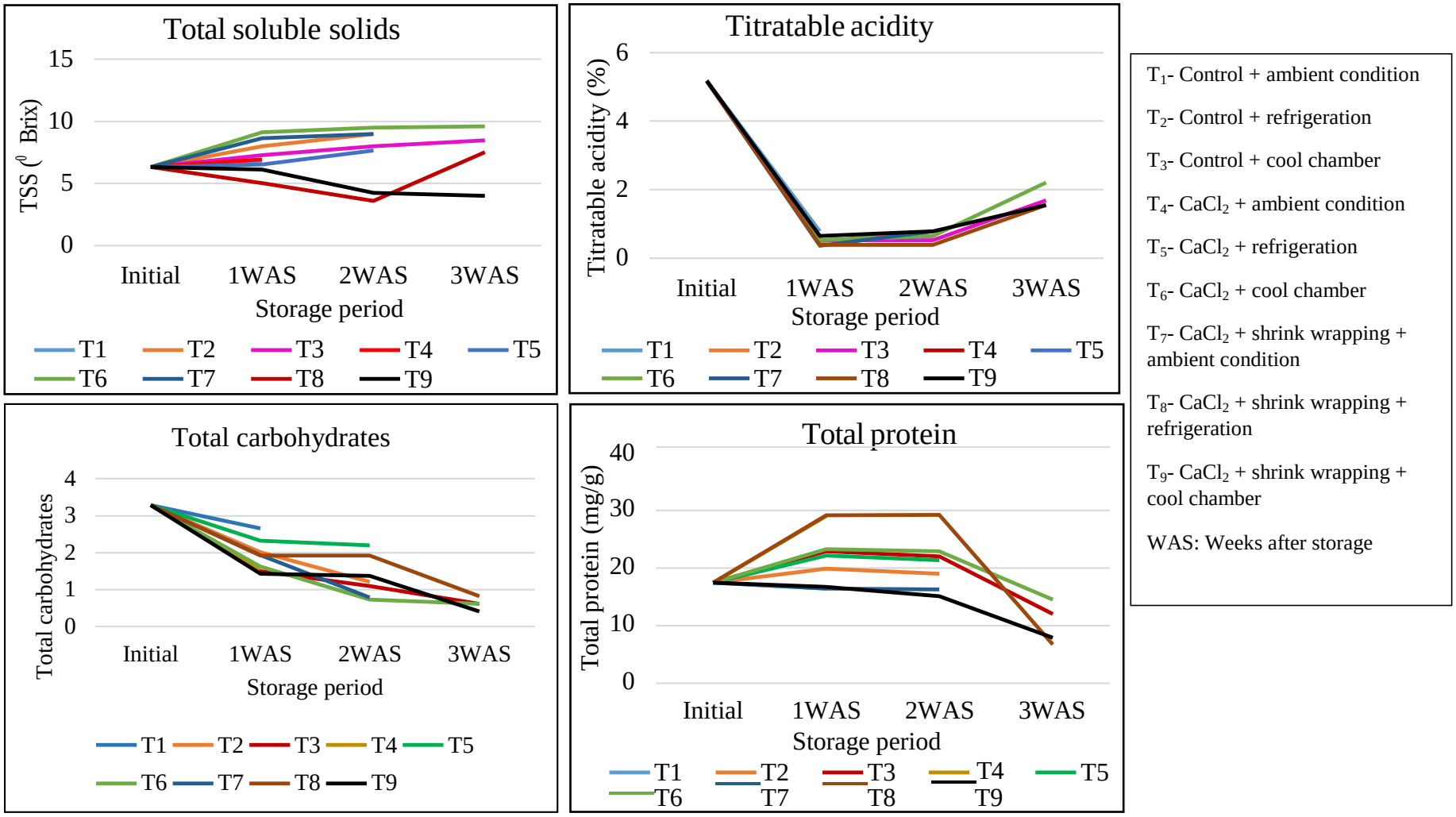
Liu *et al*. (1999) described the carbohydrate fluctuations especially in soluble sugars and starch in Hass avocado during low temperature storage and ripening. In the study it was observed that during post harvest storage at 1 or 5 ºC, glucose, fructose, sucrose and D-mannoheptulose sugar concentrations and starch decreased. Blakey *et al*. (2012) observed a slight increase in the concentration of glucose and fructose to 1.4 mg g^{-1} DW and 0.8 mg g^{-1} DW, respectively, and sucrose concentration was 1.5–4.0 mg g^{-1} DW, remained with moderate variations during ripening.

5.2.2.4. Total protein

Total protein content in the avocado fruits increased after one week of storage indicated the lower rate of enzymatic reactions and decreased towards the end of storage, which might be due to the gradual increase in proteolytic enzymatic reactions and hydrolytic degradation of proteins in the fruits (Fig. 8a). Avocado fruits had an initial total protein content of 1.75 g/100g and significantly highest value (2.92 g/100g) was observed in calcium chloride pre-treated, shrink packaged fruits under refrigeration (T_8) after 1 and 2 weeks of storage and in calcium chloride pre-treated fruits under cool chamber (T_6) after 3 weeks of storage.

Kanelli *et al*. (1989) reported the increase of proteolytic and hydrolytic enzymes such as cellulase, polygalacturonase and acid phosphatase with the progress of ripening. Castaneda-Saucedo *et al*. (2014) reported average protein content of avocado fruit, expressed in wet basis as 1.51 g/100g, with 1.49 g/100g in irrigated crops and 1.52 g/100g in rainfed crop. Blakey *et al*. (2012) observed an increase in the concentration of total soluble proteins in avocado fruits during ripening from 17.8 mg g^{-1} DW to 34.8 mg g^{-1} DW.

Fig. 8a. Effect of shrink packaging and storage temperature on biochemical parameters of avocado during storage



T_1- Control + ambient condition

T_2- Control + refrigeration

T_3- Control + cool chamber

T_4- $CaCl_2$ + ambient condition

T_5- $CaCl_2$ + refrigeration

T_6- $CaCl_2$ + cool chamber

T_7- $CaCl_2$ + shrink wrapping + ambient condition

T_8- $CaCl_2$ + shrink wrapping + refrigeration

T_9- $CaCl_2$ + shrink wrapping + cool chamber

WAS: Weeks after storage

5.2.2.5. Vitamin C

Vitamin C is water-soluble and highly susceptible to the biochemical reactions and thermic degradation and oxidative degradation during processing and storage. Ascorbic acid content of avocado fruits before storage was about 14.67 mg/100g and it decreased initially and increased with the end of storage. Initial decrease might be the result of biochemical and metabolic reactions in the fruits during pretreatment and storage conditions. While subsequent increase in vitamin C might be due to the oxidative response at low temperature storage and subsequent production of antioxidant vitamin C.

It varied non-significantly after 1 week of storage. Significantly higher ascorbic acid content was recorded in fruits kept as control in cool chamber (T_3) and calcium chloride pre-treated fruits in cool chamber (T_6) after two and three weeks of storage, respectively (Fig.8b.). Low vitamin C in control and calcium chloride pre-treated fruits under refrigeration might be due to chilling injury and increase in biochemical reactions, resulted in the loss of water soluble vitamins.

Pathirana *et al*. (2013) elucidated antioxidant activity of vitamin C due to its potential to interact enzymatically and non-enzymatically with reactive oxygen radical. In the study, vitamin C content in avocado fruits treated with 1-MCP and stored in low O_2 conditions declined during storage with higher values observed in treated samples than control. As an antioxidant compound, vitamin C might be synthesised as an oxidative stress response at low-temperature storage (Galani *et al*., 2017). Rana and Siddiqui (2018) mentioned that the decrease in vitamin C during storage was due to the oxidation of ascorbic acid by ascorbic acid oxidase enzyme and the higher retention of ascorbic acid in individual wrapped fruits of guava was observed up to 8 days of storage.

5.2.2.6. Total phenols

Total phenolic compounds increased in avocado fruits during 3 weeks of storage period (Fig.8b.), which might be due to the increase in oxidative reactions and subsequent increase in antioxidant potential phenolic compounds. Initial total phenol content was 36.50 mg/100g which increased significantly to 65.00 mg/100g in calcium chloride pre-treated fruits under ambient condition (T_4) after 1 week of storage and further to 70.00 and 85.83 mg/100g after 2 and 3 weeks, respectively in calcium chloride pre-treated, shrink packaged fruits under refrigeration at 4-7 ºC (T_8).

Vieites *et al*. (2012) reported an increase in total phenolic content of avocado cv. 'Fuerte' until 9 days in both ambient (56 µg GAE.100g^{-1}) and refrigerated (47.5 µg GAE.100g^{-1}) storage conditions and further decrease was the result of the loss of fruit mass and concentrating of molecules associated with senescence. Rana and Siddiqui (2018) observed higher phenolic content in individual shrink wrapped guava fruits compared to the control by 8 days of storage due the retention of phenols in the fruits during storage by delaying the ripening and reducing PPO activity which might oxidise phenols.

5.2.2.7. Total fat

Initial total fat content of 1.90 % increased after one week of storage in all the treatments except calcium chloride pre-treated, shrink packaged fruits under ambient storage (T_7) and in cool chamber (T_9), and decreased towards the end of storage (Fig.8b.). Throughout the storage, significantly highest total fat content was observed in calcium chloride pre-treated fruits stored in cool chamber (T_6). Initial increase in fat content might be due to the osmotic effect of calcium chloride which resulted in the leach out of water molecules from the sample and subsequent increase in lipid component of avocado fruit tissues. The gradual increase in

oxidative deterioration of lipid component might result in the decrease of total fat in avocado towards the end of storage.

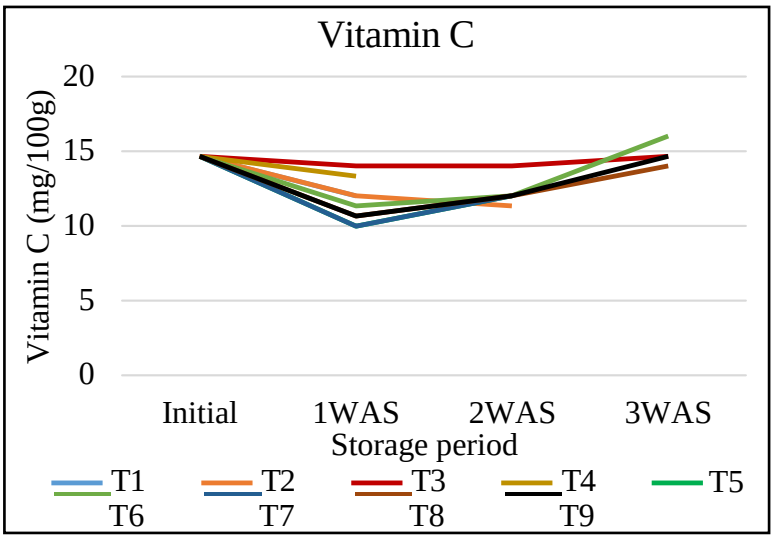
The increase in total lipid component in avocado is associated with the decrease in water content (Eaks, 1990). Castaneda-Saucedo *et al*. (2014) reported average fat content of avocado fruit in wet basis as 19.96 g/100g with 20.06 g/100g in irrigated crops and 19.85 g/100g in rainfed crop. Liu *et al*. (2019) reported that the storage temperature and time affected the fatty acid content in peanut by the oxidative hydrogenation of free fatty acids and subsequent decrease in polyunsaturated fatty acids.

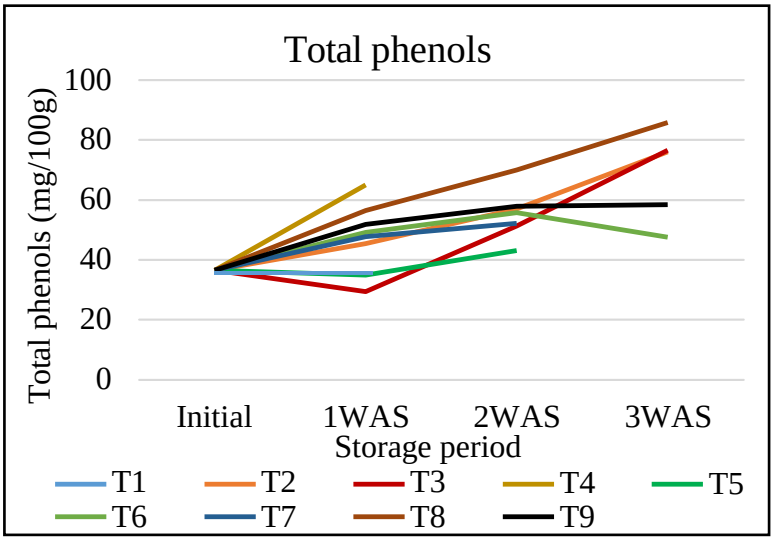
5.3. Effect of food additives on quality of frozen avocado during storage

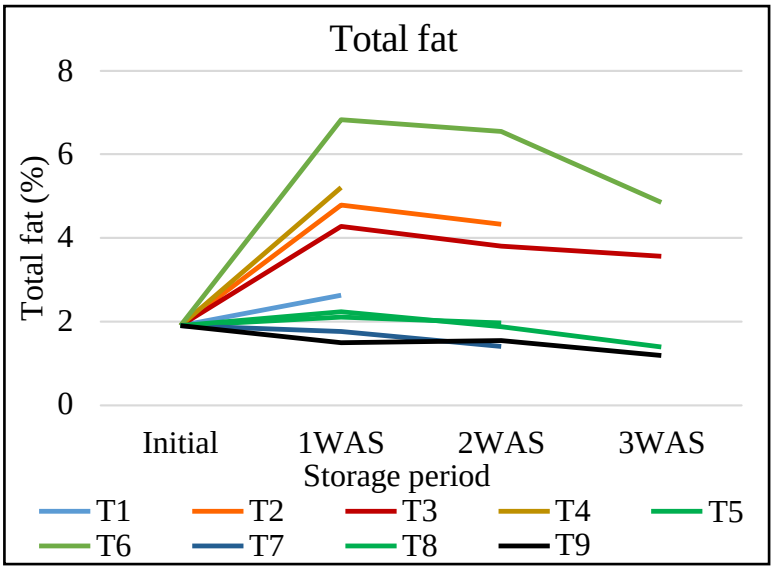
5.3.1. Total Soluble Solids

Total soluble solids increased up to one month of storage and thereafter, decreased throughout the remaining storage period which might be due to the osmotic potential of sucrose solution. Fruit slices pre-treated with 40 % sucrose added with potassium metabisulphite (T_7) (26.00 ºBrix), potassium sorbate (T_9) (23.93 ºBrix) and

Fig. 8b. Effect of shrink packaging and storage temperature on biochemical parameters of avocado during storage







T_1- Control + ambient condition
T_2- Control + refrigeration
T_3- Control + cool chamber
T_4- $CaCl_2$ + ambient condition
T_5- $CaCl_2$ + refrigeration
T_6- $CaCl_2$ + cool chamber
T_7- $CaCl_2$ + shrink wrapping + ambient condition
T_8- $CaCl_2$ + shrink wrapping + refrigeration
T_9- $CaCl_2$ + shrink wrapping + cool chamber
WAS: Weeks after storage

sodium benzoate (T_8) (22.60 ºBrix) had the significantly highest TSS during 1, 2, and 3 months after storage, respectively, which might be due to the higher concentration of sucrose (Fig.9a.).

Khan *et al.* (2014) reported increase in total soluble solids in strawberry treated with sucrose solution at 30 and 40 ºBrix along with preservatives sodium benzoate and potassium sorbate at 0.05 % concentration during 3 months storage at ambient temperature. Brochier *et al*. (2019) reported higher gain in the soluble solids of Kiwi fruit pieces treated in sucrose solution with higher osmotic concentration at 65 ºBrix. Inversion of polysaccharides and added sucrose into simpler soluble substances during storage might increase total soluble solids (Smitha and Sreeramu, 2019).

5.3.2. Titratable acidity

Titratable acidity decreased during storage with proportionate increase in the concentration of sucrose. Significantly lower titratable acidity was noticed in avocado fruit slices pre-treated with 40 % sucrose and potassium sorbate (T_9) after 2 (0.26 %) and 3 (0.19 %) months of storage, respectively (Fig.9a.).

Higher acidity would lower consumer acceptability of avocado fruit (Knapp, 1965). Presence of acidulants enhanced chemical and microbiological stability of the processed avocado products (Pauker *et al.,* 1992). Khan *et al.* (2014) reported increase in titratable acidity in strawberry treated with sucrose solution at 30 and 40 ºBrix along with preservatives sodium benzoate and potassium sorbate at 0.05 % concentration during 3 months storage at ambient temperature and the highest percent increase was observed in untreated fruits. Nardos and Wakgari (2016) mentioned that higher concentration of sucrose and storage under low temperature resulted in increased sugar content and decreased titratable acidity in avocado fruits slices.

5.3.3. Total protein

Total protein decreased during storage, followed by an increase towards the end of storage and significantly higher values (0.857, 0.782 and 1.43g/100g) were found in fruit slices pre-treated with 40 % sucrose and KMS (T_7) throughout the storage (Fig.9a.). The increase in proteolytic enzyme reactions and hydrolytic degradation of proteins in the fruit samples might result in the decrease of total proteins during storage.

Reduction in total protein content of fruit slices might be due to utilisation of proteins for metabolic activities. Heat shock proteins protect and retain other proteins and enzyme systems during heat treatment or cold storage (Hofman *et al*., 2002). Blakey *et al*. (2012) reported that ripening process in avocado resulted in the increase of protein. Sikora *et al*. (2013) mentioned that changes in protein of fruits under frozen storage conditions were non-significant in blackthorn fruits. Liu *et al*. (2019) mentioned that protein in the peanuts oxidised and decomposed into amino acids during storage.

5.3.4. Vitamin C

Vitamin C content increased in the initial phase of storage and decreased towards the end of storage (Fig.9a). Initial increase in vitamin C might be due to the pre-treatment of fruit slices with ascorbic acid and the stress response during freezing might result in the biosynthesis of vitamin C because of its antioxidant potential. 40 % sucrose and KMS (T_7) and sodium benzoate (T_8) had significantly higher vitamin C content after one and two months of storage, which might be due to the lower rate of biochemical reactions in the fruits slices and oxidative degradation of ascorbic acid into dehydroascorbic acid.

De Ancos *et al*. (2000) reported that freezing slightly increased vitamin C since damage to the fruit caused by the freezing process released vitamin C in raspberries. Khan *et al.* (2014) reported decrease in ascorbic acid of strawberry fruits treated with sucrose solution at 30 and 40 ºBrix along with preservatives sodium benzoate and potassium sorbate at 0.05 % concentration during 3 months storage at ambient temperature. Giannakourou *et al*. (2020) mentioned that osmodehydrofreezing process with a mixture of sucrose/glucose/fructose significantly decreased vitamin C owing to a mild leakage of water-soluble compounds out of the cell tissue.

5.3.5. Total phenols

Total phenols increased up to two months of storage followed by a decrease towards the end of storage after three months (Fig.9a.). Significantly higher values were observed in fruit slices pre-treated with 30 % sucrose and KMS (T_4) (96.67 mg/100g) and 30 % sucrose and potassium sorbate, (T_6) (98.30 mg/100g) after 1 and 2 months of storage, respectively, which indicates the antioxidant potential of the sample. Lower total phenolic content was observed in fruit slices treated with 40 % sucrose and KMS (T_7) after 1 month of storage and 20 % (T_3) and 40 % sucrose (T_9) with potassium

sorbate after 2 months of storage. Lower phenolic content in the fruit slices associated with the lower rate of browning of the fruit tissue during storage.

Initial increase in total phenol content in avocado fruit slices might be due to the rupture of fruit tissue, which resulted in the release of polyphenols during preparation of frozen avocado slices. Chaovanalikit and Wrolstad (2004) mentioned that some reduction might occur in phenolic content due to the reduction in enzymatic activity of polyphenol oxidase. Nowacka *et al*. (2019) reported that osmodehydration in sucrose solution for about 72 hours led to decrease of phenolic compounds.

5.3.6. Total carbohydrate

Total carbohydrate in frozen avocado slices decreased during storage except an increase after two months of storage (Fig.9a.). Fruit slices pre-treated with 40 % sucrose and KMS had significantly higher total carbohydrate with an increase to 10.17 g/100g after one month and 40 % sucrose added with KMS, sodium benzoate and potassium sorbate had significantly higher total carbohydrate after two months of storage, might be due to the higher concentration of sucrose in the samples.

An initial increase in carbohydrate might occur as the result of intake of solids and release of water from fruit samples that were stored in osmotic solution (Sikora *et al*., 2013). Decrease in total carbohydrate during storage might be due to the conversion of complex starch or carbohydrates into simple compound due to the biochemical and metabolic reactions (Yahia, 2019).

5.3.7. Total fat

Total fat content increased after one month of storage and thereafter, decreased towards the end of storage period. Frozen slices pre-treated with 20 % sucrose and KMS (T_1) and 30 % sucrose and potassium sorbate (T_6) had the significantly lower and higher fat content during storage, respectively (Fig.9b.).

Initial increase in fat content might be due to the leaching out of water from the sample immersed in osmotic solution and subsequent increase in fat content in the fruit tissues. Further decrease of fat content during storage might be due to the oxidative deterioration of lipid component in the fruit tissues. It was confirmed in the findings of Mepba *et al*. (2008) in avocado paste. The cessation in the amount of accumulated

sugars and TSS can be correlated with the accumulation of oil in fruits during postharvest storage (Liu *et al*., 1999) and hence *vice versa*.

5.3.8. Peroxide value

The lowest peroxide value was observed in frozen fruit slices pre-treated with 40 % sucrose and KMS (T_7) throughout the storage, which indicated the reduction in oxidation and subsequent rancidity and retention of flavour. Significantly lower peroxide values were obtained in all the samples preserved with potassium metabisulphite (Fig.9b.).

The activity of peroxidase enzyme decreased with the maturation of the fruit and increased with temperature and time during storage (Murasaki, 2009). Cano *et al*. (1998) reported that freezing does not diminished peroxidase activity but increased its solubility which resulted in the development of off-flavours in frozen papaya slices. Donadon *et al*. (2012) reported an increase in the POD value in Hass avocado fruit under ambient storage from 0.652 to 3.916 µmoles of H_2O_2 g^{-1} min^{-1} within 18 days of storage indicated higher oxidation of phenolic compounds while at 2 ºC, it remained unchanged.

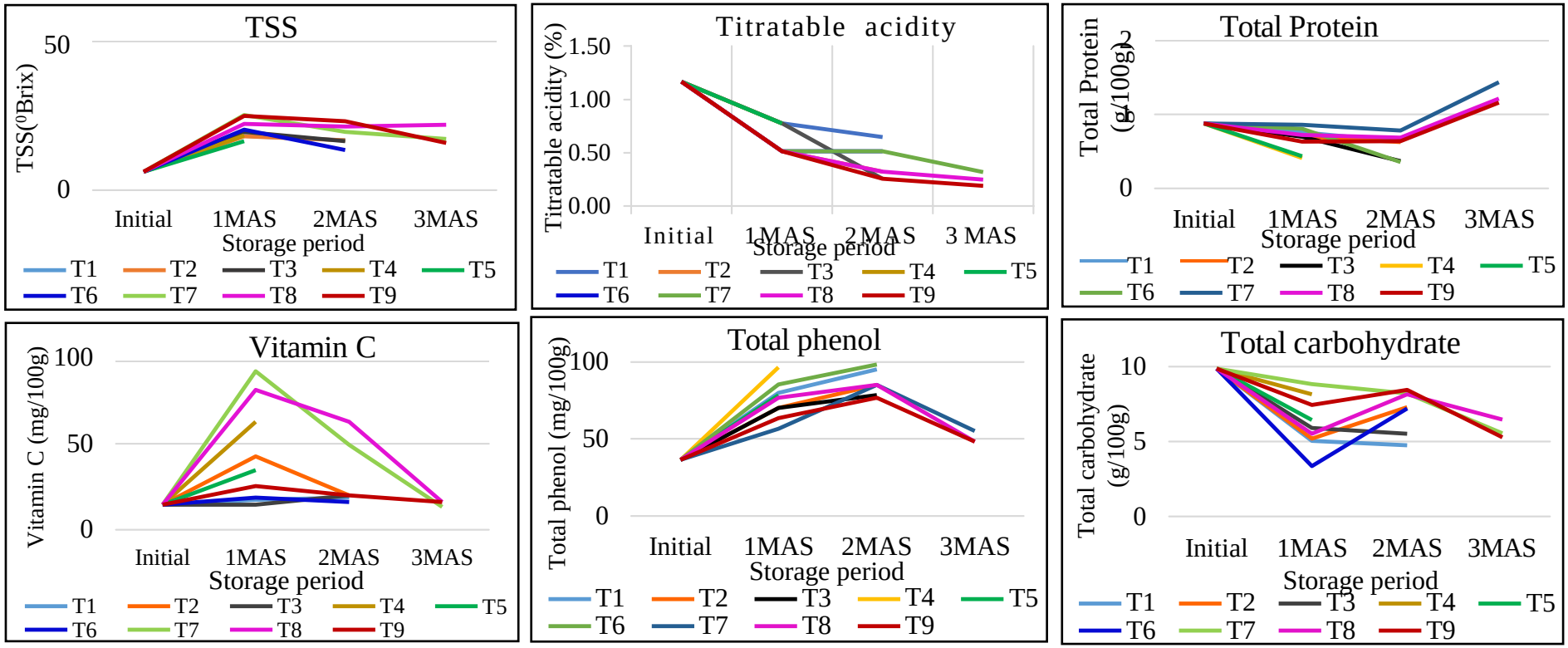
5.3.9. Polyphenol oxidase activity

Polyphenol oxidases act as catalysts for the reaction forming quinones which condensed in brown or reddish-brown colour. Polyphenol oxidase activity increased during storage due to the increased enzymatic reactions and subsequent browning in the samples and significantly lower values were observed in fruit slices pre-treated with higher concentration of sucrose (Fig.9b.).

Polyphenol oxidase activity in avocado fruit can be reduced from 100 to 20 % by decreasing the pH from 4.8 to 3.5 but the reduction in pH below 4.5 decrease the acceptability of the produce (Knapp, 1965). With a residual PPO activity less than 45 % and storage at 5 ºC maintained an acceptable colour in hydrostatic pressure treated avocado puree for at least 60 days (Lopez-Malo *et al*., 1998).

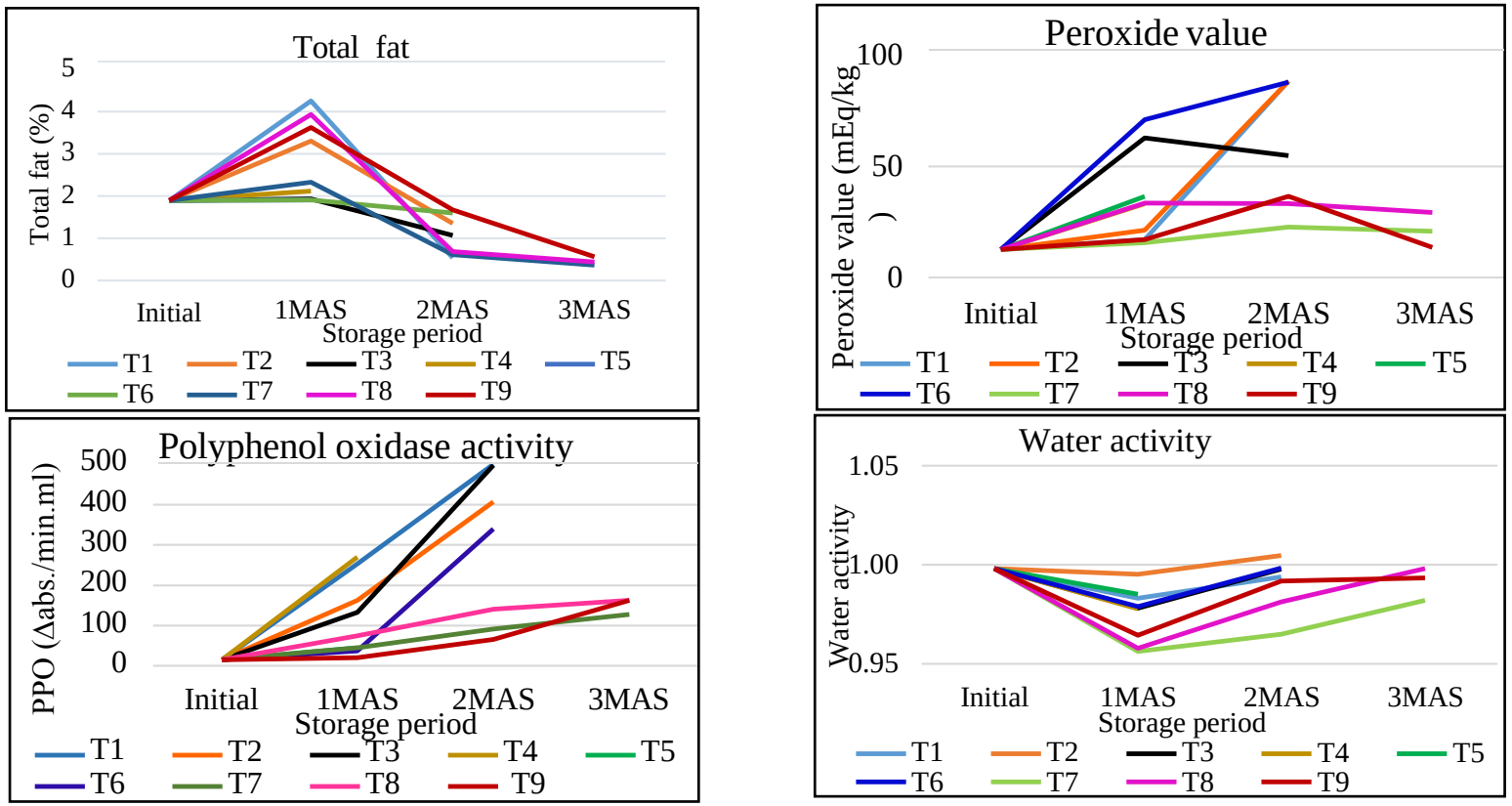
Donadon *et al*. (2012) reported that PPO activity remained unchanged as 0.871 µmoles $mg^{-1}min^{-1}$ in 'Hass' avocado fruits stored at 2 ºC but increased to 1.81 µmoles $mg^{-1}min^{-1}$ under ambient condition after 9 days of storage which is related to the oxidative browning.

Fig.9a. Effect of food additives on biochemical qualities of frozen avocado during storage



T_1- Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%)
T_2- Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
T_3- Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%)
T_4- Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
T_5- Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%)
T_6- Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
T_7- Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%)
T_8- Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
T_9- Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%)
AA - Ascorbic acid KMS - Potassium metabisulphite
NaB - Sodium benzoate KS - Potassium sorbate MAS - Months after storage

Fig. 9b. Effect of food additives on biochemical qualities of frozen avocado during storage



T_1- Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%)
T_2- Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
T_3- Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%)
T_4- Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
T_5- Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%)
T_6- Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
T_7- Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%)
T_8- Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
T_9- Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%)
AA - Ascorbic acid KMS - Potassium metabisulphite
NaB - Sodium benzoate KS - Potassium sorbate MAS - Months after storage

5.3.10. Water activity

Water activity of the frozen avocado slices decreased after one month of storage, might be due to the osmotic effect on the fruit slices and thereafter it increased towards the end of storage. Throughout the storage, lower water activity was noticed in fruit slices pre-treated with 40 % sucrose and KMS (T_7) (Fig.9b.).

In sucrose solution, water flowed out from the product to the solution and solute transferred from the solution to the product, which might contribute to the initial decrease in water activity (Shehzad *et al*., 2016; Brochier *et al.,* 2019). As the product deteriorated, moisture content increased and thereby water activity also increased.

5.3.11. Microbial population

Bacterial population was not detected in fruit slices pre-treated with 40 % sucrose added with KMS (T_7) and sodium benzoate (T_8) throughout the storage. Yeast population was absent in fruit slices pre-treated with 40 % sucrose and sodium benzoate (T_8) and lower fungal population was noticed in fruit slices pre-treated with 40 % sucrose and potassium sorbate (T_9) during three months of storage.

Frozen fruits are generally considered safe, with reduced water activity and microbial growth and thereby reduced the spoilage caused by microbial activity (De Ancos *et al*., 2000). The use of antioxidants such as ascorbic acid ensured microbial safety by decreasing the pH on cut fruit surface of avocado (Bower and Dennison, 2003). Dawson *et al*. (2020) mentioned

that freezing transferred liquid water into ice, which reduced microbial, enzymatic and lipid oxidative reactions in the samples.

5.3.12. Organoleptic evaluation

After one month of storage, higher organoleptic scores for attributes such as appearance, colour, odour, flavour and after taste were observed in fruit slices pre-treated with 40 % sucrose and potassium sorbate (T_9) and during two and three months, fruit slices pre-treated with 40 % sucrose added with KMS (T_7) had higher organoleptic scores for all sensory attributes (Fig.10.).

Bower and Dennison (2005) reported that frozen cut and ready to eat avocado portions maintained good appearance for about 6 months with some discernible

problems such as fruit browning and loss of texture. Khan *et al.* (2014) reported decrease in organoleptic qualities such as colour, texture, flavour and overall acceptability in strawberry fruits treated with sucrose solution at 30 and 40 ºBrix along with preservatives sodium benzoate and potassium sorbate at 0.05 % or 0.1 % concentration during 3 months storage at ambient temperature. Change in texture might be due to cell rupture or conversion of starch to simple sugar. Giannakourou *et al*. (2020) mentioned that the osmodehydration prior to freezing could reduce the structural collapse of frozen foods.

5.3.13. Cost of production

The total cost for the preparation of one kilogram frozen avocado fruit slices pre-treated with 40 % sucrose, 0.5 % ascorbic acid and 0.1 % KMS (T_7) and packed in 200 gauge LDPE pouches was about Rs. 450.23/-.

Freezing had more energy consumption than other conventional preservation methods, but it required only short processing time and ensured better product quality and thus overall cost for freezing can be kept lower than any other food preservation method (Barbosa-Canovas *et al*., 2005) Osmotic pretreatments had substantial effect in saving cost of energy due to the energy efficiency in freezing as well as in drying and by ensuring product quality during processing and storage (Tortoe, 2010).

5.4. Effect of food additives on quality of avocado pulp and avocado fruit powder

5.4.1. Process standardization for preparation and storage of avocado pulp

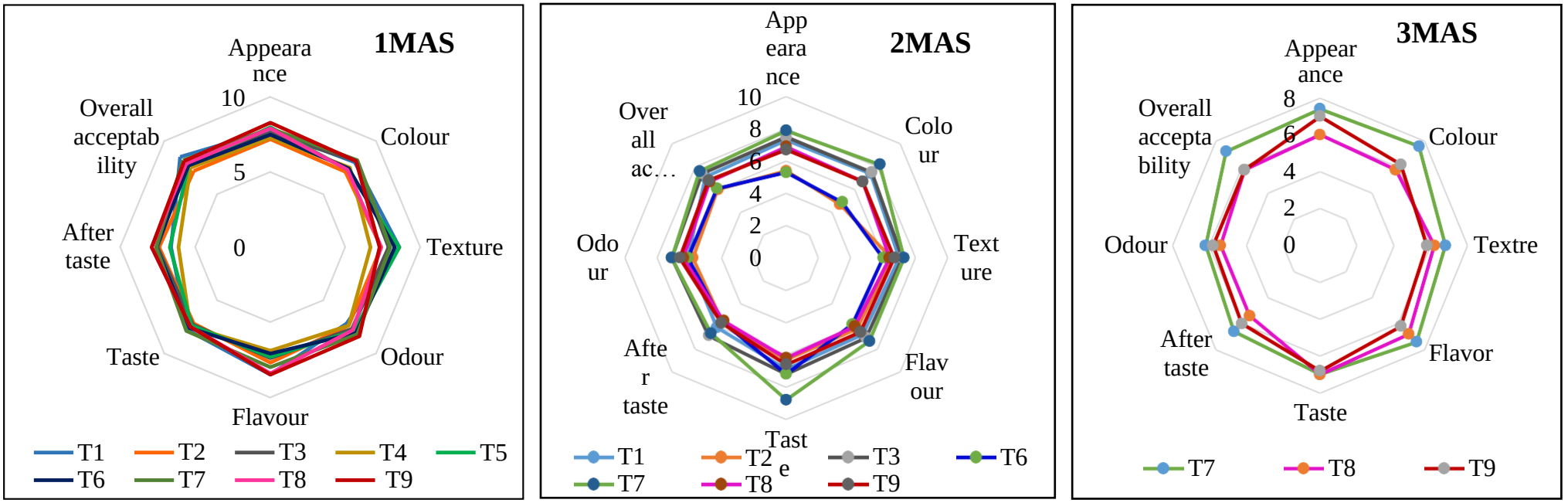
5.4.1.1. Pulp yield

Average pulp yield from avocado fruits obtained in the present study was about 71.42 %. Avocado fruit of "Hass" variety from Mexico was reported with an average pulp yield of about 79.23 % (Salvador-Reyes and Paucar-Menacho, 2019) and Castaneda-Saucedo *et al*. (2014) reported 71.39 % avocado pulp yield and Mujaffar and Dipnarine, (2020) reported 71.20 %.

5.4.1.2. Total Soluble Solids

Total soluble solids of avocado fruit pulp decreased during storage with a highest value in fruit pulp pre-treated with citric acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_6) (7.60 ºBrix) 1 month after storage. Presence of acidulants and the gradual loss of soluble solids by degradation of fruit pulp during storage might result in the decrease of TSS (Fig.11a.).

Fig. 10. Effect of food additives organoleptic scores of frozen avocado slices during storage



T_1- Avocado slices + sucrose (20%) + AA (0.5%) + KMS (0.1%)
T_2- Avocado slices + sucrose (20%) + AA (0.5%) + NaB (0.1%)
T_3- Avocado slices + sucrose (20%) + AA (0.5%) + KS (0.1%)
T_4- Avocado slices + sucrose (30%) + AA (0.5%) + KMS (0.1%)
T_5- Avocado slices + sucrose (30%) + AA (0.5%) + NaB (0.1%)
T_6- Avocado slices + sucrose (30%) + AA (0.5%) + KS (0.1%)
T_7- Avocado slices + sucrose (40%) + AA (0.5%) + KMS (0.1%)
T_8- Avocado slices + sucrose (40%) + AA (0.5%) + NaB (0.1%)
T_9- Avocado slices + sucrose (40%) + AA (0.5%) + KS (0.1%)
MAS: Months after storage
* - Unmarketable

A: Appearance C: Colour Te: Texture F: Flavour Ta: Taste At: After taste O: Odour Oa: Overall acceptability

Bishnoi *et al*. (2016) reported that sodium benzoate at 500 ppm was effective in maintaining TSS of strawberry fruit pulp at low temperature storage (7±2 ºC). It was mentioned that the low TSS content might be due to the slow rate of conversion of cell wall constituents into soluble sugars at low temperature. Minh *et al*. (2019) reported an increase in brix from 5.07 to 7.26 throughout the storage up to one month and decreased thereafter.

5.4.1.3. Titratable acidity

Titratable acidity decreased towards the end of storage in ascorbic acid treated pulp. After one month of storage titratable acidity increased in all the treatments except T_4 (fruit pulp treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature) and it had significantly lower value. Increase in acidity might be due to the formation organic acids during storage and also contributed by the acidulants present in the fruit pulp (Fig.11a.).

Zhou *et al*. (2002) mentioned that the acidity of avocado pulp indicated the degree of oxidation and rancidity of the sample during storage. Salvador-Reyes and Paucar-Menacho (2019) reported acidity of avocado pulp on initial day of storage at 0.46 to 0.51 % which increased during storage and lower values for acidity were observed in samples subjected to low temperature for longer period of time than those to high temperature.

5.4.1.4. Total protein

Total protein content of avocado pulp increased one month after storage and it decreased towards the end of storage. Significantly higher protein content was observed in T_{12} (fruit pulp treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) after 1 and 2 months of storage. The synergetic action of ascorbic acid and KMS along with protective storage condition of the treatment might prevent the enzymatic and hydrolytic deterioration of protein in the fruit pulp (Fig.11a.).

Owusu (2012) reported crude protein content of 1.72 g in fresh avocado fruit while it was 1.39 g in spread. Castaneda-Saucedo *et al*. (2014) reported non-significant difference in the total protein content of non-freeze dried avocado fruit pulp grown under rainfed condition with 5.96 g/100g while under irrigated crop it was about 6.25 g/100g in dry basis. The protein content in avocado pulp grown in rainfed and irrigated conditions were reported as an average of 1.52 and1.49 g/100g in wet basis, respectively (Castaneda-Saucedo *et al*., 2014).

5.4.1.5. Vitamin C

Vitamin C content in avocado fruit pulp increased after one month of storage and decreased towards the end of storage and it remained non-significant among the treatment throughout the storage. The decrease in vitamin C might be due to the oxidation of ascorbic acid into dehydroascorbic acid (Fig.11a.).

Vitamin C contents of processed avocado pastes treated with ascorbyl palmitate and propyl gallate ranged from 20.6-22.8 mg/100g, which increased significantly with increase of ascorbyl palmitate concentrations (Mepba *et al*. 2008).

5.4.1.6. Total phenols

Significantly higher total phenol content was noticed in fruit pulp treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature (T_{12}) after 2 months of storage. Total phenols in fruit pulp increased one and three months after storage and decreased two months after storage. The initial increase might indicate the antioxidant potential of ascorbic acid and citric acid while at the end of storage phenolic compounds might release to prevent the oxidative deterioration (Fig.11a.).

Daiuto *et al*. (2011) mentioned that phenolic compounds in fruits were responsible for the antioxidant potential. Pathirana (2013) opined that the higher antioxidant activity in avocado pulp indicated higher phenolic compounds which increased under ambient storage and decreased in cold storage.

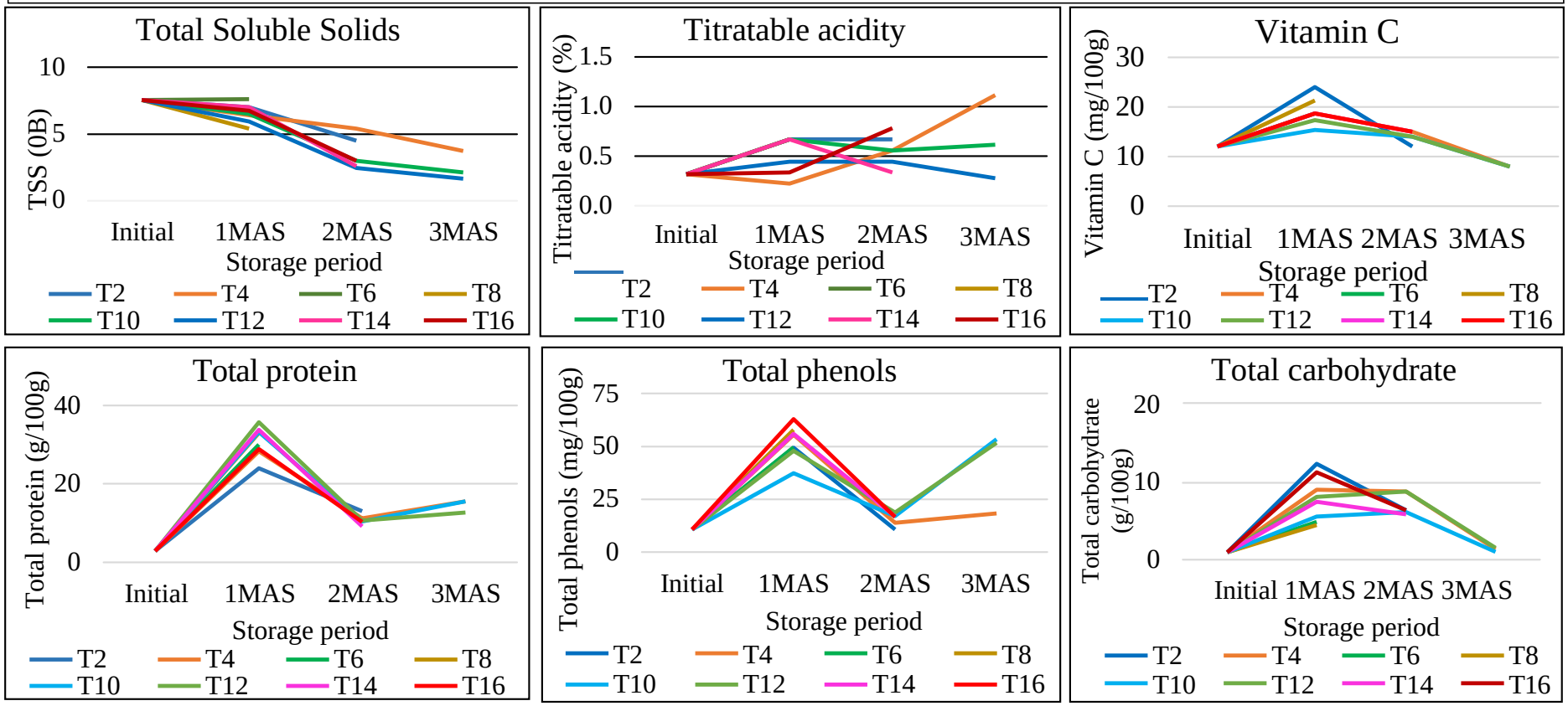
Wang *et al*. (2012) reported that the harvest period of avocado had significant effect in the total phenols content during storage which increased during storage up to 35 days in fruits harvested from January to March while in fruits harvested during April to June, total phenols increased up to 21 days and decreased thereafter. Arampath and Dekker (2019) reported that polyphenol compounds released during pulping by the disruption of cellular matrix in the fruit.

5.4.1.7. Total carbohydrates

Total carbohydrate content increased after one month of storage and decreased afterwards. Significantly higher value of total carbohydrate was seen in T_4 (fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature) and T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) after 2 months of storage (Fig.11a.).

Owusu (2012) mentioned that avocado fruit spread (20.12 g) was rich in carbohydrate than the fresh fruit (5.34 g) with the incorporation of sugar and gum.

Fig.11a. Effect of food additives on biochemical qualities of avocado pulp during storage



T_2 - Pulp + MD (2%) +LDPE bags +RT
T_4 - Pulp + MD (2%) +glass jars +RT
T_6 - Pulp + MD (3%) +LDPE bags +RT
T_8 - Pulp + MD (3%) +glass jars +RT
T_{10} - Pulp + MD (4%) + LDPE bags +RT
T_{12} - Pulp + MD (4%) +glass jars +RT
T_{14} - Pulp + MD (5%) + LDPE bags +RT
T_{16} - Pulp + MD (5%) + glass jars +RT
MD– Maltodextrin
RT – Refrigerated temperature

Mooz *et al*. (2012) reported total carbohydrate content in the range of 7.30 to 11.54 g.100 g^{-1} in different varieties of avocado fruit pulp.

5.4.1.8. Total fat

Total fat content increased during storage and higher values were observed in fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature (T_{10}) and fruit pulp per-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_{14}) after 1 and 2 months of storage, respectively (Fig.11b.).

Owusu (2012) reported that the fat content in avocado fruit spread (10.08g) was significantly lower than that in fresh fruit (15.83g). Castaneda-Saucedo *et al*. (2014) reported about 68.78 and 71.39 g/100g total fat content in non-freeze dried avocado pulp in rainfed and irrigated conditions, respectively without any significant difference.

5.4.1.9. Polyphenol oxidase activity

Polyphenol oxidase activity increased during storage which indicated the increase of enzymatic browning in fruit pulp. Significantly lower polyphenol oxidase activity was observed in fruit pulp pre-treated with citric acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_6) and also in all the pulp pre-treated with ascorbic acid after one month of storage. This might be due to the higher antioxidant activity of ascorbic acid to reduce the enzymatic browning of fruit pulp. After two months of storage, lower PPO activity noticed in fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_{14}) (Fig.11b.).

Siriphanich and Kader (1985) reported that in lettuce low O_2 and high CO_2 concentrations in Modified Atmosphere Packaging (MAP) inhibited polyphenol oxidase activity and thereby reduced browning. Cenobio-Galindo *et al*. (2019) reported higher PPO activity in control (0.29) and 50 % (0.21) and 25 % (0.24) nanoemulsion treated avocado fruit which increased to 0.33, 0.26 and 0.26 % respectively during storage.

Vieites *et al*. (2012) observed a decrease in PPO activity of avocado cv. 'Fuerte' with higher values under room temperature storage than refrigeration which was due to the influence of species characteristics, cultivating conditions and the stage of maturation, regardless of temperature. Bi *et al*. (2015) mentioned that disruption of cell wall of fruit by extraction and homogenisation decreased the particle size which facilitated phenolic compound degradation and higher PPO activity, consequently.

5.4.1.10. Peroxide value

Peroxide value of avocado fruit pulp increased during storage and lowest value was observed in fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature (T_4) after one month of storage (Fig.11b.).

Peroxide value indicated the level of autoxidation, the oxidative degradation of fatty acids which was slow at low temperature and can be hastened by heating. Autoxidation of unsaturated fatty acids forms hydroperoxides and further decompose to form aldehydes, ketones, esters and carboxylic acids, result in rancidity and develop off-flavour and unpleasant taste in fruit pulp.

According to Codex Alimentarius (1993) food grade and fresh oils have a peroxide values less than 10 meq O_2/kg and the rancidity was 20-40 meq O_2/kg. Owusu (2012) reported that as the storage period increased, peroxide value also increased which was in a slower pace in refrigerated samples than those in ambient storage. Chaiyasut *et al.* (2019) reported that the peroxide value of manually and mechanically extracted oils of avocado was 7.96 and 8.03 meq O_2/kg, respectively which increased after 90 days of storage in transparent glass bottles.

5.4.1.11. Water activity

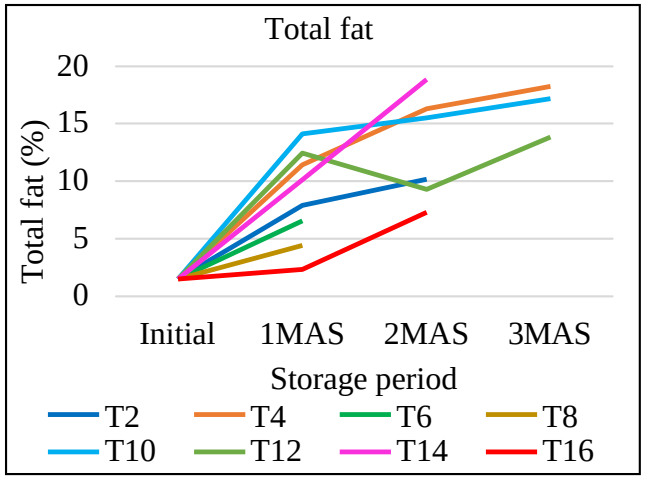
Water activity increased up to one month of storage and thereafter it remained without much variation. Lower water activity was seen in fruit pulp pre-treated with citric acid and KMS, packed in LDPE bags and stored in ambient temperature (T_2) and fruit pulp pre-treated with ascorbic acid and KMS, packed in LDPE bags and stored in refrigerated temperature (T_{10}) after one month of storage. Lowest water activity may ensure the longer storage life of samples especially by reducing the microbial infection and biochemical reactions (Fig.11b.).

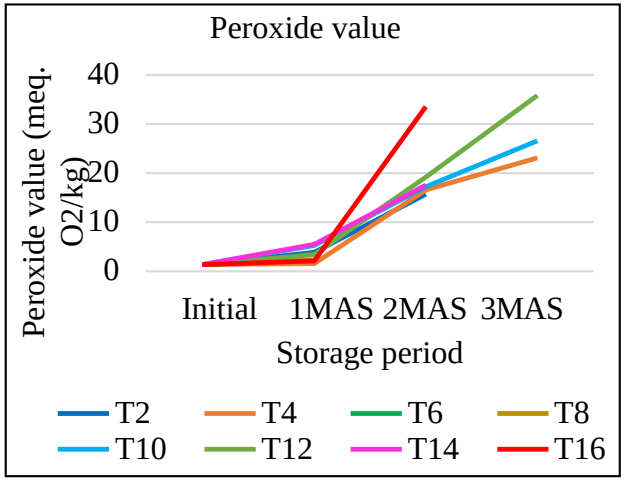
Iturriaga *et al*. (2002) reported water activity of 0.980 and 0.990 in avocado pulp and processed guacamole, respectively which is consistent with the present study. Cortes-Rodríguez *et al*. (2019) reported water activity of 0.971 in avocado pulp added with additives such as antioxidants (vitamin C and tocopherol) and preservatives (potassium sorbate and sodium benzoate), used for the preparation of guacamole. Nayaka *et al*. (2020) reported water activity of avocado fruit pulp as 0.691.

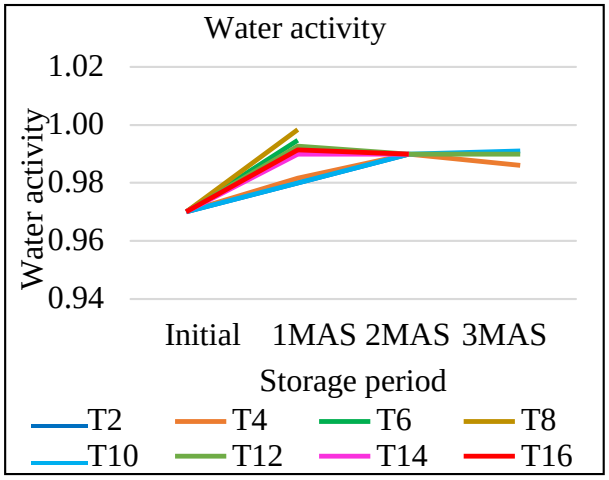
5.4.1.12. Viscosity

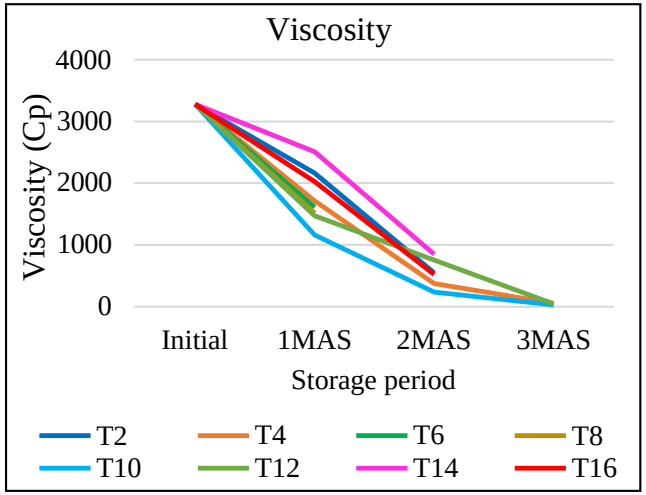
Viscosity decreased during storage which might relate with the rise in water activity and loss of consistency of the fruit pulp. Significantly higher viscosity was observed in fruit pulp pre-treated with ascorbic acid and sodium benzoate, packed in

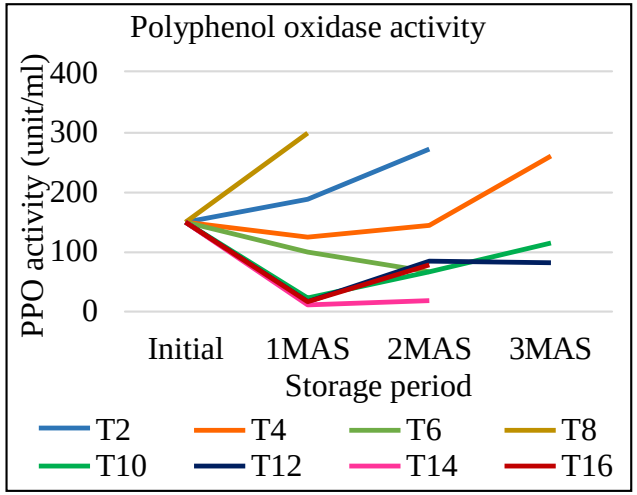
Fig.11b. Effect of food additives on biochemical qualities of avocado pulp during storage











T_2 - Pulp + MD (2%) +LDPE bags +RT
T_4 - Pulp + MD (2%) +glass jars +RT
T_6 - Pulp + MD (3%) +LDPE bags +RT
T_8 - Pulp + MD (3%) +glass jars +RT
T_{10}- Pulp + MD (4%) + LDPE bags +RT
T_{12}- Pulp + MD (4%) + glass jars +RT
T_{14}- Pulp + MD (5%) + LDPE bags +RT
T_{16}- Pulp + MD (5%) + glass jars +RT
MD– Maltodetrin
RT – Refrigerated temperature

LDPE bags and stored in refrigerated temperature (T_{14}) after 1 and 2 months of storage, respectively (Fig.11b.).

Kassim and Workneh (2020) mentioned that the decrease in elasticity of the ripen fruit resulted in the increase in viscosity of fruit pulp. Bi *et al*. (2015) reported that increase in viscosity was due to the decrease in particle size and increase in puree to water ratio. Ikhuoria and Maliki (2007) observed that lower viscosity indicated increase in unsaturation which contributed to the important attribute of preventing the dryness of skin. Cortes-Rodríguez *et al*. (2019) mentioned that rheological property indicated the food quality influencing consumer acceptance and the release of flavours, and in guacamole viscosity was reported in the range of 1773.3 to 10776.7 cP.

5.4.1.13. **Microbial load**

Microbial population was very less up to one month of storage and it increased towards second and third months after storage. After two months of storage, fungal population was absent in T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature) and T_{16} (fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in glass jars and stored in refrigerated temperature), bacterial and yeast population was nil in T_2 (fruit pulp pretreated with citric acid and KMS, packed in LDPE bags and stored in ambient temperature) and T_{16} (fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in glass jars and stored in refrigerated temperature). The lowest microbial count of bacteria, yeast and fungi were observed in T_{16} (fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in glass jars and stored in refrigerated temperature) up to two months and in T_{10} (fruit pulp pre-treated with ascorbic acid and KMS, packed in LDPE bags and stored in refrigerated temperature) after three months of storage.

Pauker *et al*. (1992) mentioned that acidulants ensured chemical and microbiological stability of frozen avocado salad base, in which bacterial quality was major concern because of the consumption of nonsterile and uncooked product. Owusu (2012) reported a steady increase in the microbial population to a peak and declined to the end of storage which occurred slowly in refrigerated samples than those under ambient storage. In samples under ambient storage, the peak was observed at 34.2 cfu/mL microbial population with a shelf life of 37.49 days and in refrigeration it was observed with a microbial population of 20.52 cfu/mL, having 47.50 days of shelf life. Kassim and Workneh (2020) noticed conducive environment for mould development in fruits within the package and stored under ambient condition.

5.4.1.14. Organoleptic evaluation

Organoleptic quality of the fruit pulp decreased during storage due to rancidity and enzymatic browning. After one month of storage, higher total scores were recorded in colour and appearance and after two months of storage, it was for texture followed by colour and appearance. At the beginning of storage, organoleptic scores for all the sensory attributes not varied among the treatments except the lower values of after taste in fruit pulp pre-treated with citric acid. During the storage period, higher scores for most of the sensory attributes were observed in T_{10} (fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature) and T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature).

After three months of storage, among treatments such as T_4 (fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature), T_{10} (fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature) and T_{12} (fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature), higher total scores were recorded for appearance and colour (Fig.12.).

Pathirana *et al*. (2013) reported that during postharvest storage avocado fruit pulp was highly sensitive to oxidative process, mainly autoxidation which resulted in the development of carbonyl compounds responsible for undesirable flavours and degradation of quality.

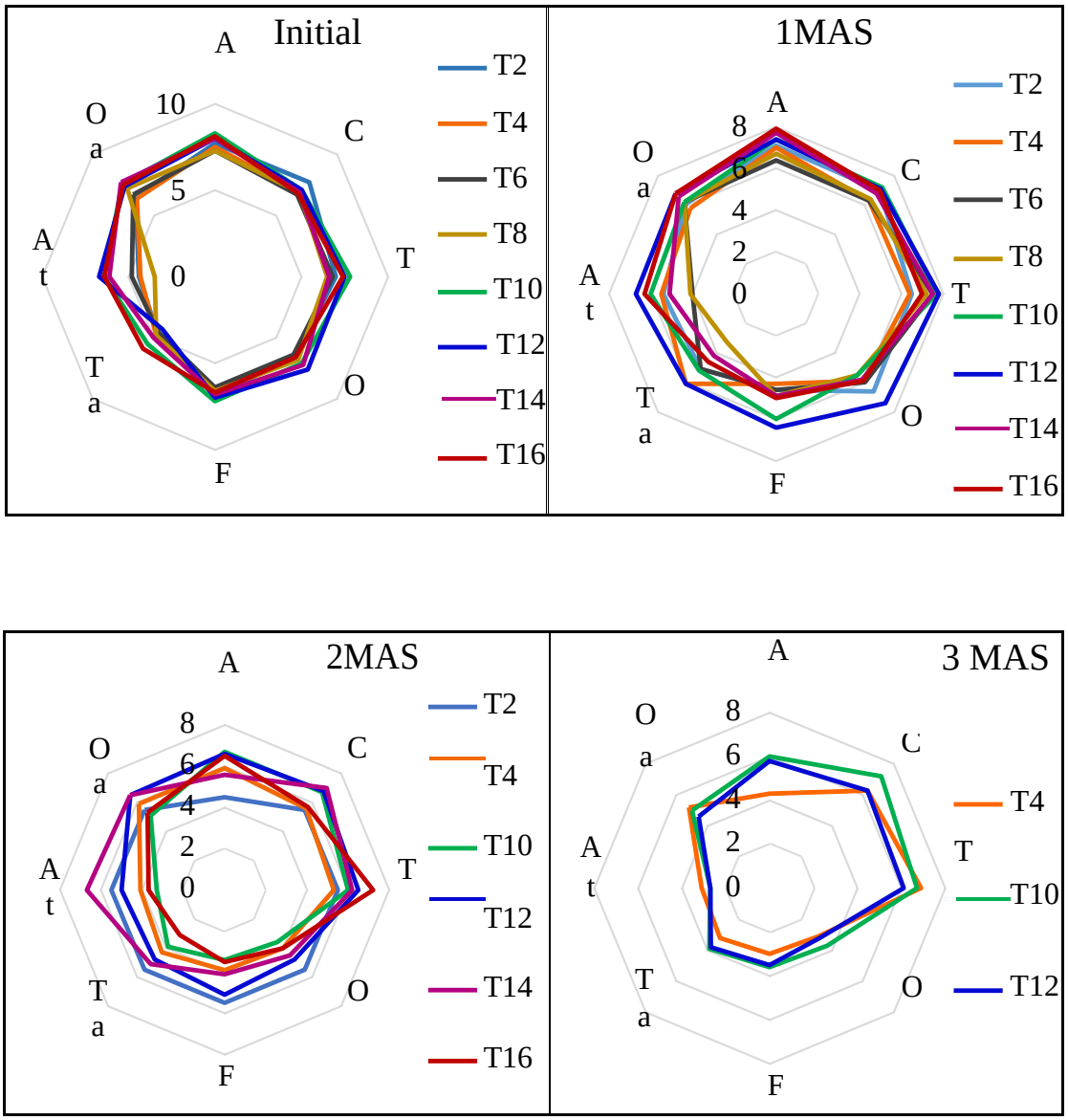
Thermal treatments modified naturally soft and spreadable avocado pulp into a lumpy and slightly watery paste and resulted in bitter tastes and unpleasant odours, causing lower scores by consumers which were mainly due to oxidation of lipids (Salvador-Reyes and Paucar-Menacho, 2019).

5.4.1.15. Cost of production

The cost required for the preparation and storage of 1 kg avocado pulp added with 0.5 % ascorbic acid, 0.1 % KMS, packed in LDPE pouches or glass jars stored in refrigeration for one month was about Rs. 339.98/-.

Meera (2019) calculated the cost for 1 kg avocado fruit spread added with KMS, citric acid and sugar as Rs.550.00. According to Niir Project Consultancy Services (NPCS), cost of production for one litre pulpy fruit drinks such as mango, orange and

Fig. 12. Effect of food additives on organoleptic scores of avocado pulp during storage



T_2 - Pulp + CA +KMS + LDPE bags + LT T_4 - Pulp + CA +KMS + glass jars + LT
T_6 - Pulp + CA +NaB + LDPE bags + LT T_8 - Pulp + CA +NaB + glass jars + LT
T_{10}- Pulp + AA +KMS + LDPE bags + LT T_{12} - Pulp + AA +KMS + glass jars + LT
T_{14}- Pulp + AA +NaB + LDPE bags + LT T_{16}- Pulp + AA +NaB + glass jars + LT
MAS: Months after storage A: Appearance C: Colour T: Texture
F: Flavour Ta: Taste At: After taste O: Odour Oa: Overall acceptability

pineapple juice was reported as Rs. 134.83. Pokharkar *et al*. (2017) reported the processing cost of one litre mango drink with a purchase rate of Rs 40 per one kilogram of mango was Rs. 54.20.

5.4.2. Optimization of process conditions for preparation of avocado fruit powder

In the present study, freeze dried powder yield of avocado fruit pulp was 38.57 %. Garcia *et al*. (2008) reported that the amount of freeze dried avocado powder was about 30 to 35 % relative to the mass of avocado pulp applied. According to the report of Mujaffar and Dipnarine (2020), the yield of dried pulp from fresh pulp was about 17.10 % for freeze-dried avocado samples.

5.4.2.1. Bulk density

Bulk density increased after one month of storage and decreased towards the end of storage, which might occur due to the increase in moisture content with the end of storage and subsequent aggregation of powder. Higher bulk density was observed in samples with increased concentration of high molecular weight additives like maltodextrin due to its bulking properties and the potential to reduce moisture absorption of the powder (Fig.13).

Dantas *et al*. (2018) reported bulk density of spray dried avocado fruit powder in the range of 0.377 to 0.511 g/cm^3. Avocado powder with high fat content on the surface, resulted in adherence of the particles together or agglomerate and decreased bulk density. Bulk density of freeze dried instant green smoothie powder and freeze dried pumpkin powder were 0.47±0.0424 g/mL (Dilrukshi and Senarath, 2020) and 0.113±0.0006 g/mL (Caliskan and Dirim, 2015), respectively. The mean value of bulk density of vacuum dried mango powder added with maltodextrin and tricalcium phosphate was 537.2 kg.m^{-3} (Jaya *et al*., 2006). Mujaffar and Dipnarine (2020) reported a lower bulk density of 0.16 g/mL in freeze dried avocado powder, which required more volume to occupy, than oven-dried. Chauhan and Singh (2020) reported bulk density of 0.492 ±0.14 g/cm^3 in freeze dried butter fruit milk shake powder contained avocado pulp, pasteurized toned milk, sugar and maltodextrin.

5.4.2.2. Solubility

Solubility of freeze dried avocado powder decreased gradually towards the end of storage and significantly higher solubility was observed in freeze dried fruit powder pre-treated with 4 and 5 % maltodextrin after two months of storage. Crystalline nature of the maltodextrin might enhance the solubility of fruit powder. After three months of

storage, significantly higher solubility was noticed in freeze dried fruit powder pre-treated with 3 % maltodextrin, packed in glass jars and stored in low temperature (T_8) (Fig.13.).

Marulanda *et al*. (2018) reported solubility per cent in the range of 39.90 to 86.80 % in spray dried avocado powder which decreased with the increase in concentration of maltodextrin. It was elucidated that the higher concentration of maltodextrin increased the particle size and cohesiveness of aggregating particle which indicated the diffusion of water into the particles. Cortes-Rodríguez *et al*. (2019) reported solubility of 71.20 % in freeze dried avocado powder. Mujaffar and Dipnarine, (2020) reported that freeze dried avocado powder was insoluble with a water solubility of 11.4 ± 3.9 % in which the addition of water resulted in the formation of thick paste. Adetoro *et al*. (2020) reported that crystalline nature of the powder added with maltodextrin provided higher solubility of 96.50 %.

5.4.2.3. Hygroscopicity

Hygroscopicity increased during storage and it decreased with the increase in concentration of maltodextrin which might be due to the anticaking property of the maltodextrin which reduced the moisture absorption of fruit powder. Significantly lower values were observed in T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) after 1 and 2 months of storage and in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) after 3 months of storage (Fig.13.).

Hygroscopicity of vacuum dried mango powder added with maltodextrin (1.61 g) was 1.8 times effective than that of tricalcium phosphate (0.89 g) and it was observed that the hygroscopicity increased with decreasing amount of maltodextrin and tricalcium phosphate. Average hygroscopicity of vacuum dried mango powder added with maltodextrin and tricalcium phosphate was 6.4 % (in the range of 5.3 to 10.3 %) (Jaya *et al*., 2006).

Marulanda *et al*. (2018) reported 10 % hygroscopicity in spray dried avocado powder which decreased with the increase of drying additives such as maltodextrin in the formulation. It was observed that solubility decreased with exposure to temperature by the low affinity for water due to the interactions between fat and maltodextrin. Stępien *et al*. (2020) mentioned that to reduce the hygroscopicity of food powders, high molecular weight biopolymers and carrier agents like maltodextrin could be added which improved drying of plant materials.

5.4.2.4. Colour values (L, a, b)

L value decreased towards the end of storage and increase in a* value during storage indicated the change in green colour of the powder to red colour. Colour value b* increased during storage, indicated the decrease in yellow colour of avocado powder. Higher L* values and lower a* and b* of the samples with higher maltodextrin content indicated the better appearance of freeze dried fruit powder (Fig.13.).

Owusu (2012) reported that in avocado spread the yellowish-green colour faded slowly in refrigerated samples than those under ambient storage with the increase in storage days. It indicated the faster degradation of samples at high temperature with high oxygen availability than lower temperature. Illeperuma and Nikapitiya (2015) reported a reduction in L* value from 15.5 to 14.2 in avocado slices packaged in MAP with different thickness, stored at 8 ºC and 90±2 % RH for 10 days.

Vieites *et al*. (2012) observed high luminosity in 'Fuerte' avocado pulp in the range of 80.8 to 88.8 which decreased during storage and negative a* values representing the predominance of green colour in the samples decreased under room temperature. While the b* value indicated the presence of yellow colour of fruit pulp, increased up to 9 days of storage under refrigeration and decreased up to sixth day under room temperature. Cenobio-Galindo *et al*. (2019) reported the brightness of avocado mesocarp decreased during storage and increase in a* indicated the loss of green colour and decrease in b* values associated with the loss of yellow colour.

Water content in fresh avocado absorbed more light and became more yellow, which might result in higher b* value (42.0 ± 1.5) than avocado powder (27.6 ± 0.3). While the L* and a* of fresh avocado (75.5 and -5.9) were also higher than avocado powder (74.8 and -5.0) respectively (Cortes-Rodríguez *et al*., 2019). Mujaffar and Dipnarine (2020) reported L*, a* and b* values for freeze-dried avocado powder were 82.96 ± 0.59, -14.32 ± 0.02 and 60.38 ± 0.29 respectively. Trujillo-Mayol *et al*. (2020) reported L*, a* and b* values of avocado fruit at different ripening stages according to the small, medium and large size fruits with respect to changes in size were in the range of 24.7 to 25.6, -0.1 to 0.2 and 0.9 to 4 respectively. Chauhan and Singh (2020) reported colour value of L*, a* and b* in freeze dried butter fruit milk shake powder as 77.62±0.69, -2.22±0.57 and 19.63±0.17 respectively.

5.4.2.5. Total Soluble Solids

TSS decreased after one and three months of storage and increased after two months of storage. Total soluble solids increased with the addition of higher concentration of maltodextrin, which is a soluble solid (Fig.14a.).

Vieites *et al*. (2012) observed a peak in soluble solids content of 'Fuerte' avocado fruits on third and sixth days of storage under room temperature and on ninth day under refrigeration, indicated the use of soluble solids as substrate for energy transformation in respiratory metabolism during post harvest period. Cenobio-Galindo *et al*. (2019) reported that an increase in TSS of avocado fruits treated with nanoemulsion was observed during 60 days of storage, which was lower than fruits without treatment. Dilrukshi and Senarath (2020) reported that TSS of fresh smoothie powder added with maltodextrin and soluble fiber was 22.27 ºBrix and freeze drying reduced it to 12.33 ºBrix and it was mentioned that addition of maltodextrin would increase total soluble solids by decreasing the moisture content during freeze drying.

5.4.2.6. Titratable acidity

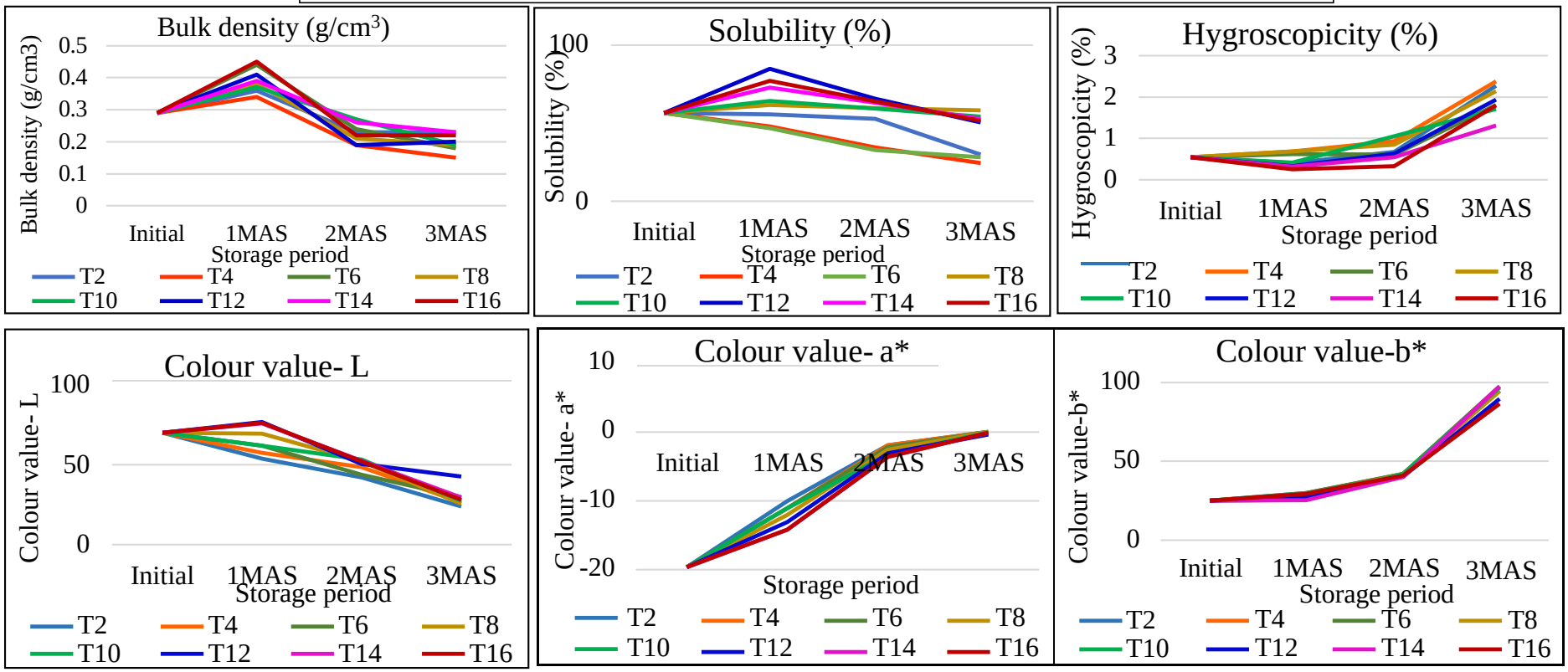
Titratable acidity varied without any significant difference among the treatments which increased after two months storage and decreased after one and three months of storage which might be due to the presence of maltodextrin which is a soluble solid (Fig.14a.).

Mujaffar and Dipnarine (2020) observed decrease in the pH and corresponding increase in acidity of freeze dried avocado powder over 12 weeks of storage which might develop bitter taste in the powder. Chauhan and Singh *et al*. (2020) reported decrease in titratable acidity of freeze dried avocado powder with the addition of maltodextrin and Ceballos *et al.* (2012) obtained similar findings in freeze-dried soursop fruit pulp.

5.4.2.7. Total protein

Total protein decreased during storage which might be due to the gradual increase in proteolytic enzyme reactions and hydrolytic degradation of protein in the powder. Significantly higher values were observed in T_8 (fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in refrigerated temperature) and T_{12} (fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in ambient temperature) after 2 and 3 months of storage, respectively (Fig.14a.).

Fig.13. Effect of food additives on physical qualities of avocado powder during storage



T_2 - Pulp + MD (2%) +LDPE bags +RT

T_4 - Pulp + MD (2%) +glass jars +RT

T_6 - Pulp + MD (3%) +LDPE bags +RT

T_8 - Pulp + MD (3%) +glass jars +RT

T_{10} - Pulp + MD (4%) + LDPE bags +RT

T_{12} - Pulp + MD (4%) + glass jars +RT

T_{14} - Pulp + MD (5%) + LDPE bags +RT

T_{16} - Pulp + MD (5%) + glass jars +RT

MD– Maltodextrin

RT – Refrigerated temperature

MAS- Months after storage

Garcia *et al*. (2008) invented the method of obtaining avocado powder in freeze drier, reported protein content which varied from 4.50 to 7.69 % during the dehydration time of 10 hours. Dantas *et al*. (2018) observed loss of protein content in powdered avocado mixtures than the fresh sample; however maltodextrin with 23 % concentration prevented the loss of protein. Castaneda-Saucedo *et al*. (2014) reported non-significant difference in the total protein content of 6.30 and 6.25 g/100g in freeze dried avocado fruit pulp grown under rainfed and irrigated condition, respectively.

5.4.2.8. Vitamin C

Vitamin C content increased after one month of storage which might be due to the pretreatment with ascorbic acid and thereafter it decreased with increase in oxidative reactions. Significantly highest values were seen in T_{14} (fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature) after one month of storage and in T_8 (fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in refrigerated temperature) after 3 months of storage. These results are additional contribution to the previous studies that reported the potential of maltodextrin in preserving bioactive compounds like vitamin C (Fig.14a.).

In a study conducted by De Ancos *et al*. (2000) on raspberry, freezing process increased vitamin C content due to damage occurred to the fruit tissues caused by ice crystals formed during freezing and further release of vitamin C. Dantas *et al*. (2018) reported that maltodextrin with 23 % concentration had positive effect on preserving bioactive compounds like ascorbic acid. Dilrukshi and Senarath (2021) reported that slight reduction (12.50 %) of vitamin C in instant smoothie powder of avocado after freeze drying which might also be due to the processing operations, but it was lower than other drying methods.

5.4.2.9. Total phenols

Total phenolic content varied without any significant difference among the treatments and decreased in all the treatments except fruit pulp pre-treated with 2 % (T_2) and 4% (T_{10}) maltodextrin packed in LDPE bags and stored in refrigerated temperature after one month of storage and increased thereafter (Fig.14a.).

Antioxidant property of phenolic compounds may attribute to the increase in its value with the end of storage at which fruit powder may undergo oxidative deterioration resulted in rancid, off flavour and discoloration.

Chaiyasut *et al*. (2019) reported that the total phenolic content of avocado oil differed non-significantly for 3 months of storage under different temperature (4, 30 and 40 ºC) in clear and amber glass bottles. Phenols are major plant compounds having antioxidant capacity due to their redox properties and ability for absorbing and neutralizing free radicals and decomposing peroxides (Alissa *et al*., 2020). Chauhan and Singh (2020) reported total phenolic content of 416.2 ± 5.04 mg/100g in freeze dried butter fruit milk shake powder.

5.4.2.10. Total carbohydrates

Total carbohydrates increased after one month and decreased towards the end of storage except a rise observed in fruit pulp pre-treated with 2 % maltodextrin packed in LDPE bags (T_2) and glass jars (T_4) and 3 % maltodextrin packed in LDPE bags (T_6), stored in refrigerated temperature. Significantly higher values were found in fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags (T_{14}) and glass jars (T_{16}) stored in refrigerated temperature, which may attribute to the higher content of maltodextrin, a polysaccharide (Fig.14a.).

Garcia *et al*. (2008) invented the method of obtaining freeze dried avocado powder and reported carbohydrates content of 7 % at 2 h which increased to 15.4 % after 10 h and peak value of carbohydrates 20.13 % was observed after 5 h during the dehydration. Decrease in the carbohydrate content in avocado paste could occur due to the leaching of water soluble constituents during storage (Mepba *et al*., 2008). Chauhan and Singh (2020) reported total carbohydrate content of 33.16 % in freeze dried butter fruit milk shake powder.

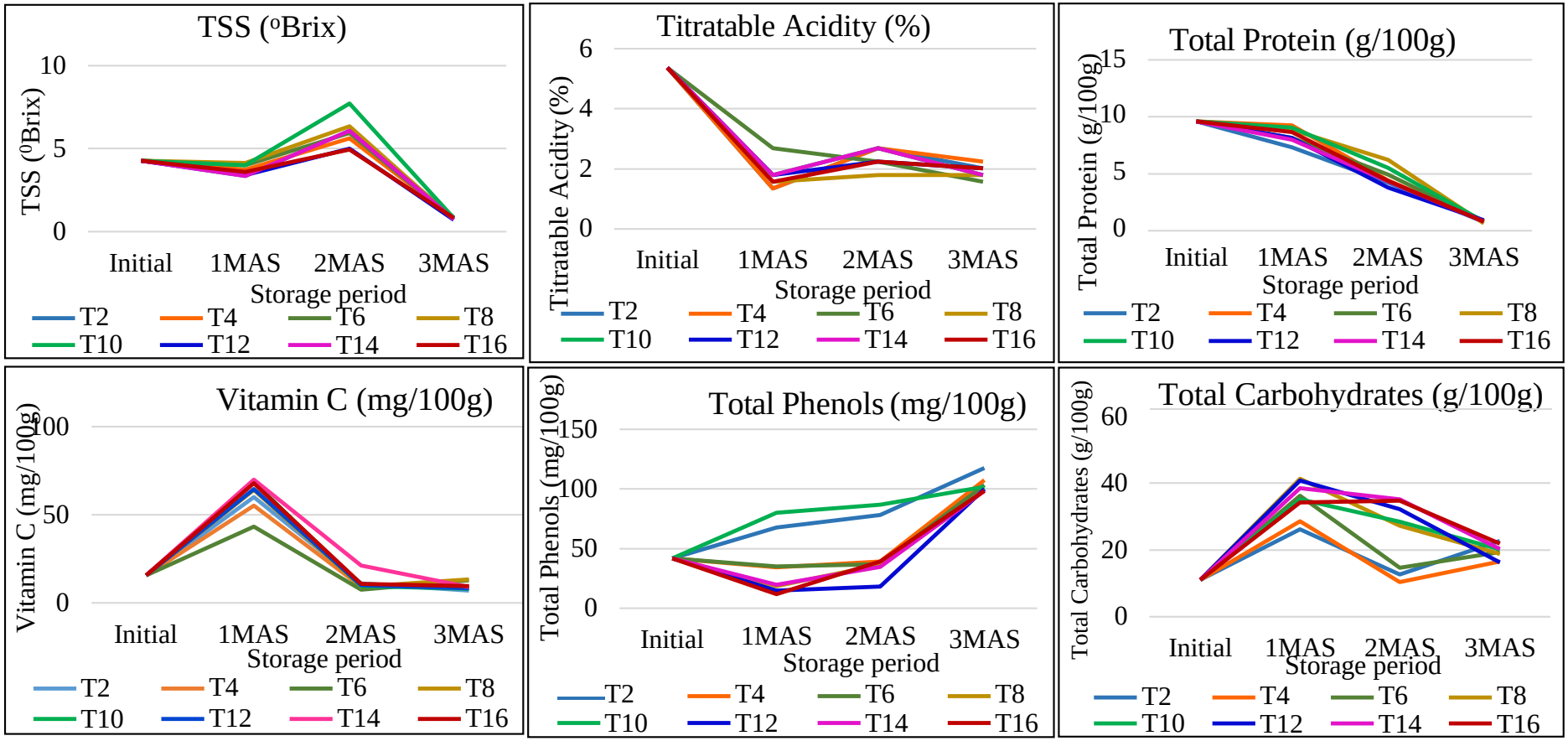
5.4.2.11. Total fat

Total fat content in freeze dried avocado fruit powder decreased during storage and significantly higher values were observed in samples with 5% concentration of maltodextrin after one and two months of storage. Decrease in total fat content may attribute to the rapid oxidative deterioration of lipids (Fig.14b.).

Castaneda-Saucedo *et al*. (2014) reported no difference in the total fat content of freeze dried and non-freeze dried avocado fruit pulp grown under rainfed condition (68.78 g/100g) while under irrigated crops it was about 71.34 and 71.39 g/100g, respectively.

Garcia *et al*. (2008) invented the method of obtaining avocado powder in freeze drier, reported a reduction in fat content in freeze dried avocado fruit powder from 66.2

Fig.14a. Effect of food additives on biochemical qualities of avocado powder during storage



T - Pulp + MD (2%) +LDPE bags +RT
2

T - Pulp + MD (2%) +glass jars +RT
4

T - Pulp + MD (3%) +LDPE bags +RT
6

T - Pulp + MD (3%) +glass jars +RT
8

T - Pulp + MD (4%) + LDPE bags +RT
10

T - Pulp + MD (4%) + glass jars +RT
12

T - Pulp + MD (5%) + LDPE bags +RT
14

T - Pulp + MD (5%) + glass jars +RT
16

MD– Maltodextrin RT – Refrigerated temperature

to 59.72 % during the dehydration time from 2 to 10 hours. Chauhan and Singh (2020) reported total fat content of 42.2±0.22 % in freeze dried butter fruit milk shake powder.

5.4.2.12. Peroxide value

Peroxide value increased during storage which indicated the oxidation and rancidity of fat with the end of storage and significantly lowest values were observed in fruit powder pre-treated with 4 and 5 % of maltodextrin after 2 and 3 months of storage (Fig.14b.).

Best performance of peroxide activity was observed in samples with high maltodextrin composition which decreased the effect of temperature on increasing the oxidation of sample matrix (Marulanda *et al*., 2018). In the stability study of avocado oil, Chaiyasut *et al*. (2019) concluded that avocado oil remained relatively stable for 3 months without any significant change in peroxide value under storage conditions of low temperature in the absence of light.

5.4.2.13. Water activity

Water activity increased gradually during storage and lower values were noticed in samples with higher concentration (5 %) of maltodextrin throughout the storage. Low hygroscopic nature of maltodextrin may contribute to the ability to reduce water activity in the powder (Fig.14b.).

Water activity is an important quality attribute indicating the shelf stability as well as stability of the freeze dried product towards lipid oxidation, enzymatic browning and hydrolytic reactions. Dantas *et al*. (2018) reported water activity of spray dried avocado powder in the range of 0.28 to 0.42 within an acceptable range of biochemical and microbial stability. It was found that maltodextrin has no significant effect on water activity of avocado powder. Water activity of instant green smoothie powder (Dilrukshi and Senarath, 2020) and freeze-dried pumpkin powder (Dirim and Calıskan, 2012) were reported as 0.172 and 0.197, respectively. Cortes-Rodríguez *et al*. (2019) reported water activity of freeze dried avocado powder as 0.257. Mujaffar and Dipnarine (2020) reported an increase in the water activity of freeze dried avocado powder after 12 weeks of storage from 0.352 to 0.534.

5.4.2.14. Microbial load

In freeze dried avocado fruit powder, microbial population was nil up to one month of storage and after two and three months of storage, lower microbial count was noticed in samples with higher concentration of maltodextrin. In all the samples, yeast population was not observed during the storage. Lower microbial population in all the

treatments of freeze dried avocado powder may be due to the lower free water availability, which ensured longer storage life and safety under proper packaging and storage conditions.

Even though the processing operations for freeze drying include peeling, cutting, blending or grinding contributed high risk of microbial growth, freeze drying had better microbial stability. It reduced the total plate count of yeast and mold from 6.99 and 8.25 log CFU/mL to 5.99 and 1 log cfu/mL, in which the reduction was reported as 85.6 % and 100 % respectively (Dilrukshi and Senarath, 2020).

5.4.2.15. Organoleptic evaluation

Organoleptic qualities decreased in all the samples and T_{16} (fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature) had highest total scores throughout the storage. In all the samples, organoleptic attributes such as

appearance, colour and texture obtained comparatively higher scores than those for odour, flavour, taste and after taste (Fig.15.).

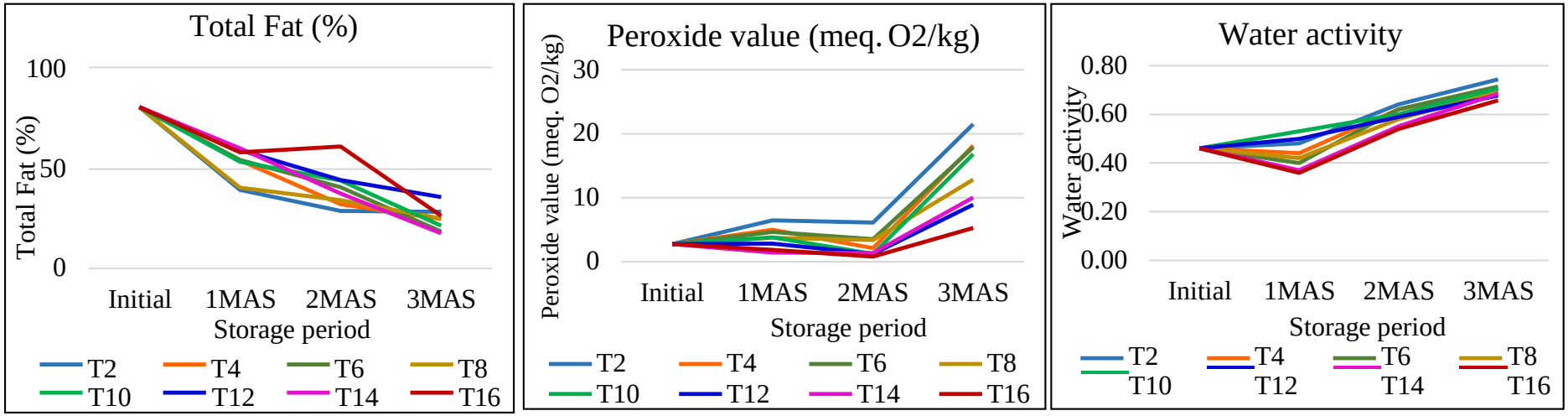
In fresh smoothie powder added with maltodextrin and soluble fiber, Dilrukshi and Senarath (2021) reported an actual likeness for odour, colour, appearance, and overall acceptability at the margin of 75 % likeness, while taste and texture obtained most acceptable level in which slightly bitter aftertaste and texture were improved by adding sugar or honey and thickening agent. Cortes-Rodríguez *et al*. (2019) reported loss of avocado flavour when the pulp was dried in microwaves and bitter tastes in spray dried avocado powder with antioxidants.

5.4.2.16. Cost analysis

Cost of production for the preparation and storage of 1 kg freeze dried avocado fruit powder was Rs. 1224.86 /-.

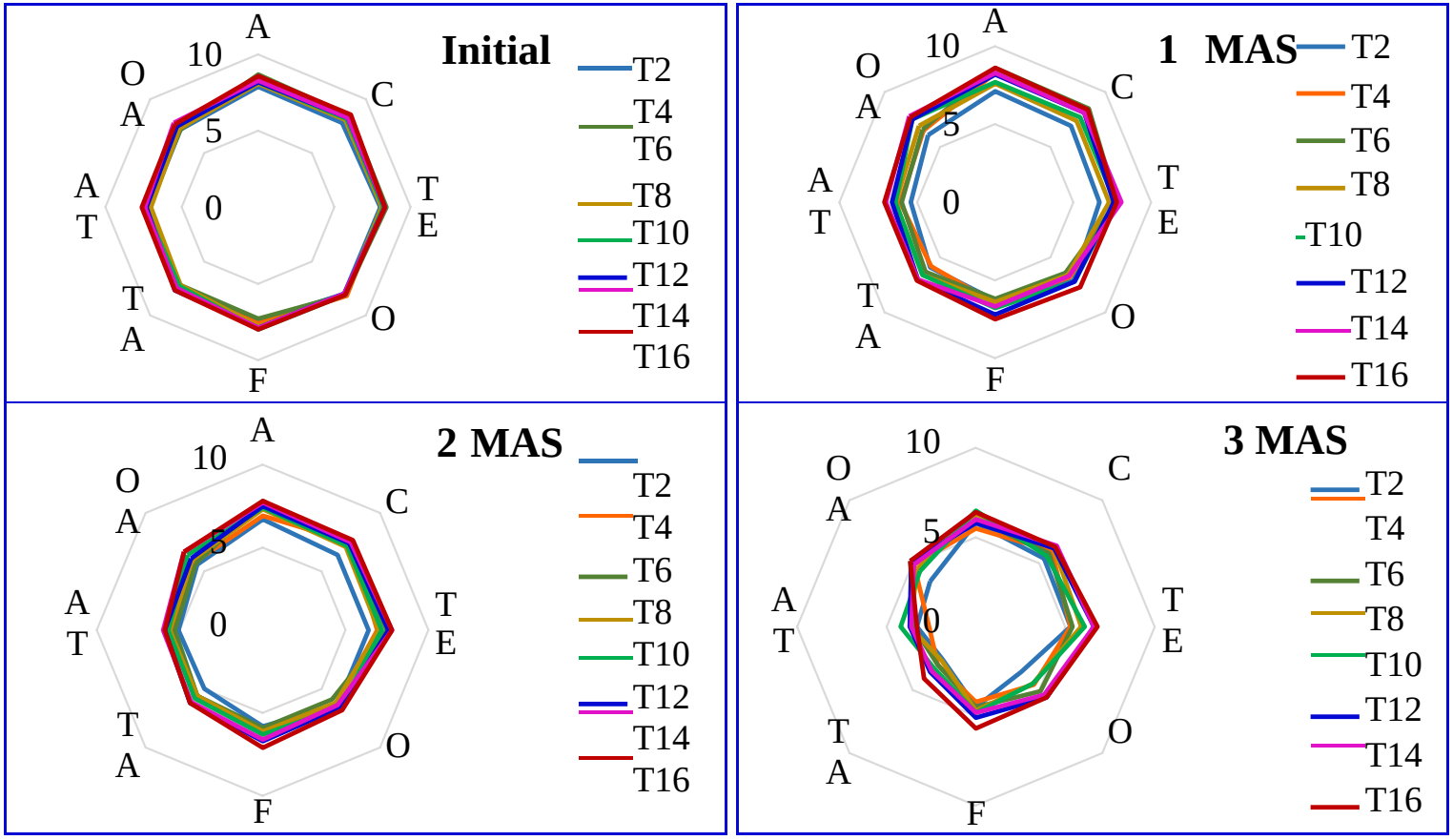
By reducing the drying time and minimizing the damage of the product, freeze drying became cost effective, also it reduced the handling, transportation and storage cost (Sagar and Kumar, 2010). Pretreatments improved the final quality, reduced energy costs and minimized colour loss during freeze drying (Ciurzynska and Lenart, 2011). In freeze drying, the high cost was attributed to high equipment cost, long drying period and high energy consumption (Mujaffar and Dipnarine, 2020).

Fig.14b. Effect of food additives on biochemical qualities of avocado powder during storage



T_2 - Pulp + MD (2%) +LDPE bags +RT	T_4 - Pulp + MD (2%) +glass jars +RT	T_6 - Pulp + MD (3%) +LDPE bags +RT
T_8 - Pulp + MD (3%) +glass jars +RT	T_{10} - Pulp + MD (4%) + LDPE bags +RT	T_{12} - Pulp + MD (4%) + glass jars +RT
T_{14} - Pulp + MD (5%) + LDPE bags +RT	T_{16} - Pulp + MD (5%) + glass jars +RT	
MD– Maltodextrin	RT – Refrigerated temperature	

Fig. 15. Effect of food additives on organoleptic scores of freeze dried avocado powder during storage



T_2- Pulp + MD (2%) +LDPE bags +LT T_4- Pulp + MD (2%) +glass jars +LT T_6- Pulp + MD (3%) +LDPE bags +LT

T_8- Pulp + MD (3%) +glass jars +LT T_{10}- Pulp + MD (4%) + LDPE bags +LT T_{12}- Pulp + MD (4%) + glass jars +LT

T_{14}- Pulp + MD (5%) + LDPE bags +LT T_{16}- Pulp + MD (5%) + glass jars +LT MAS: Months after storage

A: Appearance C: Colour Te: Texture F: Flavour Ta: Taste At: After taste O: Odour

Oa: Overall acceptability Ts: Total score

5.5. Preparation of instant fruit shake

Avocado fruit powder shake with better sensorial acceptability was prepared at 1:2:1:2 of avocado fruit powder, skimmed milk powder, sucrose and water.

Reconstitution of nutritionally rich, organoleptically stable and wholesome blended powder has great scope due to the instant preparing and easy consuming readiness especially in highly perishable fruits like avocado. Dantas *et al*. (2018) developed avocado powder drink by reconstituting spray dried avocado pulp, milk and maltodextrin blend, maintaining nutritional and sensorial qualities without any browning. Shiby *et al*. (2021) prepared avocado milkshake powder by blending milk and pulp in 3:1 ratio along with either 10% sugar or maltodextrin at different concentration of 2, 6 and 12 % followed by spray drying.

SUMMARY

6. SUMMARY

The project titled 'Post harvest characterisation and management of avocado (*Persea americana* Mill.)' was carried out in the Department of Post Harvest Technology during 2018-2021. The main objective of the study was to characterise the avocado accessions collected from various parts of Kerala and to evaluate the effect of different preservation methods in the post harvest management of avocado.

In the first experiment on characterisation of avocado genotypes, fourteen accessions were collected from the Regional Agricultural Research Station, Ambalavayal, Wayanad, twelve accession from Kanthaloor, Idukki and one from Thanniyam in Thrissur district. The twenty seven accessions collected from the subtropical and tropical areas were characterised based on horticultural traits according to 'Descriptors for avocado' by IPGRI and their biochemical attributes were also analysed.

Among the 27 accessions, fruit length varied from 5.50 to 12.86 cm, fruit diameter from 4.62 to 9.87 cm and fruit weight from 58.34 to 640.41 g. Inflated base, rounded apex, central or symmetric apex positions with smooth, glossy fruit surface were commonly seen. Wide variability was observed in the fruits of accessions collected from Wayanad while more uniformity was noticed among the accessions collected from Idukki. Light green coloured brittle fruit skin with slight adherence were mostly observed while accessions from Idukki had thicker skin than those from Wayanad. In most of the accessions, light green coloured fruit skin with greenish yellow coloured flesh next to the skin and light yellowish colour next to seed were observed. Buttery textured flesh with less fibre and neither sweet nor bitter in taste were the common characteristics of avocado. Even after 4 hours of cutting open the fruit, degree of discoloration was nil in most of the accessions.

With regard to the biochemical characterisation, total soluble solids were observed in the range of 4.27 ºBrix in Acc. 20 to 11.3 ºBrix in Acc. 13, titratable acidity ranged from 0.28 to 2.84 % in Acc 27 and Acc 12, respectively. Vitamin C was varied from 5.33 mg/100g in Acc. 18 and Acc. 20, which was the lowest, to 20 mg/100g (highest) in Acc. 11. Lowest carbohydrate was seen in Acc. 7 (0.407g/100g) and highest in Acc. 25 (3.817 g/100g). Total protein content of fruits of accessions collected from Wayanad was in the range of 0.50 to 1.59 g/100g and those from Idukki it was 8.33 to

11.93 g/100g. Total phenols content varied from 33.33 mg/100g to 102.83 mg/100g, which was higher in accessions from Wayanad. Total flavonoids varied from 24.80 to 66.67 mg/100g, which was insignificant.

Total fat content from avocado extract varied from 0.79 to 10.02 % in Acc.4 and Acc.7, while oleic acid from 13.49 to 86.86 g/100g in Acc. 6 and Acc. 10, respectively. Total ash varied from 0.27 to 1.79 % in Acc. 8 and Acc. 3, respectively. Minerals such as calcium, potassium and iron content were in the range of 4.9 to 13.46 mg/100g, 122.27 to 460.00 mg/100g and 0.04 to 0.44 mg/100g, respectively. Crude fibre content was higher (7.61 %) in Acc. 6 and lower (2.24 %) in Acc. 12. Most of the biochemical characters varied significantly at 0.05 % level with the only exception of total flavonoids, which was insignificant.

Considering the horticultural traits, accessions from Idukki were uniform in characteristics, with superior quality. Considering the biochemical characteristics, higher vitamin C, total phenols, potassium and ash contents were seen in accessions from Wayanad, and total protein and iron content were higher in accessions from Idukki. Accession with highest total carbohydrates, total flavonoids and calcium content among the 27 accessions and highest vitamin C, potassium, iron, total ash, oleic acid and crude fibre among the accessions collected from Idukki were reported in accession 25 and it was selected as superior accession.

Inihibitory concentration (IC_{50}) indicating the antioxidant scavenging activity of the accession 25, in DPPH assay was 4.07 µg/mL, in FRAP assay it was 2.58 µg/mL and in ABTS assay it was 0.10 µg/mL.

In the second experiment, effect of shrink packaging and storage temperature on quality and shelf life of avocado was investigated and the physical, physiological and biochemical parameters of the fruits were analysed. Mature fruits of avocado were surface sanitised with 2 ppm ozone and treated with 2 % calcium chloride, followed by shrink packaging and subsequently stored under ambient, refrigeration (4-7 ºC) and cool chamber (12-13 ºC).

Avocado fruits under ambient storage had storage life of 7 days while in refrigeration and cool chamber it was more than 20 days. Shrink packaging further improved the shelf life to more than 27 days. During storage, PLW increased in a faster rate in control and slower in shrink packaged fruits under refrigeration (T_8) from 0.05

to 0.89 %, followed by cool chamber (T_9) from 0.30 % to 1.28 % after 27 days of storage, respectively. Due to the climacteric pattern of respiration in avocado, climacteric peak was seen in fruits in cool chamber kept as control (T_3) (3.5 %), calcium chloride pre-treated fruits (T_6) (5.73 %) and calcium chloride pre-treated shrink packaged fruits (T_9) (2.5 %) after 9 days of storage and in calcium chloride pre-treated shrink packaged fruits under ambient storage (T_7) (7.73 %) after 3 days of storage. Lower respiration rate was noticed in fruits kept as control (T_2), calcium chloride pre-treated (T_5) and calcium chloride pre-treated shrink packaged fruits (T_8) under refrigerated storage. A peak in the ethylene evolution was also observed in all the treatments during storage and lowest ethylene evolution rate was seen in calcium chloride pre-treated fruits under cool chamber (T_6) followed by calcium chloride pre-treated shrink packaged fruits under refrigeration (T_8) and calcium chloride pre-treated shrink packaged fruits under cool chamber (T_9). Texture of avocado fruits decreased during storage and highest retention of firmness during storage was noticed in fruits under cool chamber (fruits kept as control (T_3), calcium chloride pre-treated fruits (T_6) and calcium chloride pre-treated shrink packaged fruits (T_9)) followed by fruits under refrigeration (fruits kept as control (T_2), calcium chloride pre-treated fruits (T_5) and calcium chloride pre-treated shrink packaged fruits (T_8)). Decay per cent was higher in control sample and 100 % decay was noticed within 9 days of storage. The fruits treated with calcium chloride (T_4) and shrink packaging (T_7) under ambient conditions decayed fully after 21 and 24 days of storage, respectively. Shrink packaged fruits stored under both refrigeration and cool chamber remained without 100 percent decay up to 27 days of storage indicated slow pace of decay in the fruits. Along with the physiological disorders such as grey pulp, pulp spot and mesocarp discoloration, post harvest diseases such as anthracnose, fruit rot and stem end rot also hastened the rate of decay.

Significantly higher values of total soluble solids were observed in calcium chloride pre-treated fruits under cool chamber (T_6) throughout the storage period of 3 weeks, which increased from the initial value of 6.33 ºBrix to 9.13, 9.5 and 9.6 ºBrix at 1, 2 and 3 weeks after storage, respectively. Lower titratable acidity (0.39 %) was recorded in shrink packaged fruits under refrigeration (T_8) after 2 weeks of storage and it increased towards the end of storage. Total carbohydrate in the initial period of storage was 3.29 g/100g which decreased during storage and the higher contents were seen in fruits kept as control under ambient conditions (T_1) (2.66 g/100g), calcium

chloride pre-treated fruits under refrigeration (T_5) (2.20 g/100g) and calcium chloride treated shrink packaged fruits under refrigeration (T_8) (0.82 g/100g) at 1, 2 and 3 weeks after storage respectively. Significantly higher total protein content were noticed in calcium chloride pre-treated shrink packaged fruits under refrigeration (T_8) after one (2.92 g/100g) and two (2.92 g/100g) weeks of storage and in calcium chloride pre-treated fruits under cool chamber (T_6) (1.45g/100g) after 3 weeks of storage. Vitamin C increased during storage with significantly higher values seen in fruits under cool chamber kept as control (T_3) (14 mg/100g) and calcium chloride pre-treated fruits (T_6) (16 mg/100g) after 2 and 3 weeks of storage respectively. During 3 weeks of storage, total phenols content increased and significantly higher values were seen in calcium chloride pre-treated fruits under ambient storage (T_4) (65.00 mg/100g), 1 week after storage and calcium chloride pre-treated shrink packaged fruits under refrigeration (T_8) (70.00 and 85.83 mg/100g) after 2 and 3 weeks of storage. Throughout storage higher total fat was seen in calcium chloride pre-treated fruits under cool chamber (T_6) which decreased 1 to 3 weeks after storage.

Taking into consideration of the longer shelf life, lower PLW, diminished respiration rate, lower ethylene evolution rate with better retention of firmness along with least titratable acidity, higher total carbohydrates, total protein and total phenols, calcium chloride pre-treated fruits with shrink packaging and stored under refrigeration at 4-7 ºC (T_8) was considered as the ideal pre-treatment for the storage of avocado fruit.

In experiment III, effect of food additives on quality of frozen avocado slices during three months of storage was analysed. Avocado slices added with different proportions of sucrose (20-40 %) along with ascorbic acid (0.5 %) in combination with one of the preservatives (KMS, sodium benzoate, potassium sorbate @ 0.1 %) were quick frozen in blast freezer to -20 ºC for 30 minutes and the frozen slices were packed in LDPE (200 gauge) pouches and subsequently stored under subzero condition (-18 ºC).

Fruit slices pre-treated with 40 % sucrose and KMS (T_7), potassium sorbate (T_9) and sodium benzoate (T_8) had the significantly higher TSS of 26.00, 23.93 and 22.60 ºBrix after one, two and three months of storage respectively. TSS decreased during storage, after an initial increase after one month of storage. Titratable acidity decreased during storage and significantly lower titratable acidity (0.26 %) was seen in fruit slices pre-treated with 40 % sucrose and potassium sorbate (T_9) after two months of storage.

After a steep rise in the vitamin C content during first month, it decreased towards the end of storage and fruit slices pre-treated with 40 % sucrose and KMS (T_7) (92.00 and 49.33 mg/100g) and fruit slices pre-treated with 40 % sucrose and sodium benzoate (T_8) (81.33 and 62.67 mg/100g) recorded significantly higher values at 0.05 % level difference after 2 and 3 months of storage, respectively. Total phenols increased up to 2 months and then decreased and lower values were seen in fruit slices pre-treated with 40 % sucrose and potassium sorbate (T_9) (63.33 mg/100g) one month after storage and in fruit slices pre-treated with 20 % sucrose (T_3) and 40 % sucrose (T_9) with potassium sorbate (76.67 mg/100g) two months after storage. Highest total carbohydrate was seen in fruit slices pre-treated with 40 % sucrose and KMS (T_7), one (10.17 g/100g) and two (8.20 g/100g) months after storage. After an initial increase in fat content after first month, it decreased and the higher fat content was noticed in fruit slices pre-treated with 20 % sucrose and KMS (T_1) (4.25 %) and fruit slices pre-treated with 30 % sucrose and potassium sorbate (T_6) (2.60 %) after one and two months of storage. Peroxide value increased up to 2 months and decreased afterwards with higher content in samples with 40 % sucrose. T_7 recorded lower polyphenoloxidase activity throughout the storage. Water activity increased after one month of storage and significantly lower values were noticed in fruit slices pre-treated with 40 % sucrose and KMS (T_7). Bacterial colonies were observed in the initial day of storage and increased during storage. Fungal colonies were seen after one month and yeast colonies were observed only in fruit slices pre-treated with 20 % sucrose and KMS (T_1) and fruit slices pre-treated with 40 % sucrose and KMS (T_7) after two month of storage. Fruit slices pre-treated with 40 % sucrose and KMS (T_7) had comparatively lower microbial population throughout the storage. Fruit slices pre-treated with 40 % sucrose and KMS (T_7) recorded higher organoleptic scores for appearance, colour, texture, flavour, taste, after taste and overall acceptability throughout the storage. The cost of production of 1 kg frozen avocado slices was estimated with Rs.450.23.

Considering the biochemical parameters, significantly higher TSS, vitamin C, total carbohydrate and organoleptic scores and lower polyphenoloxidase activity, water activity and microbial population, avocado slices pre-treated with 40 % sucrose added with KMS (T_7) stored at -18 ^{o}C was selected as ideal preservation technique for the frozen avocado fruit slices.

In the experiment IVa, effect of food additives on quality of avocado pulp and avocado fruit powder was evaluated. In the process of standardization for preparation and storage of avocado pulp, after surface sanitisation and pre-treatments of fruits, avocado pulp was added with citric acid and ascorbic acid (0.5 %) separately, in combination with a preservative, either KMS or sodium benzoate @ 0.1 %, packed in glass jars and vacuum packed LDPE (200 gauge) bags and stored under ambient and refrigerated conditions for 3 months.

After extraction, average pulp yield obtained was 71.42 %. TSS content decreased during storage and significantly higher value was seen in fruit pulp pre-treated with citric acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_6) (7.60 º Brix) and lowest titratable acidity was noticed in fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature (T_4) (0.22 %). In all the treatments highest protein content was seen one month after storage and significantly higher value was reported in fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature (T_{12}) after one and two months of storage. Vitamin C content varied non-significantly during storage while both fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature (T_4) and fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature (T_{12}) had highest total carbohydrate after two months of storage. Total fat content increased during storage and significantly higher content was noticed in fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature (T_{10}) (14.10 %) and fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_{14}) (18.85 %) after one and two months of storage respectively. Peroxide value increased during storage and significantly lower value was seen in fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature (T_4) (1.53 meq/kg) and viscosity decreased throughout storage with significantly higher was recorded in fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_{14}) after one (2505.6 cP) and two (846 cP) months of storage. Water activity increased up to one month of storage and thereafter remained without much variation, significantly lower water activity was observed in fruit pulp pre-treated with citric acid (T_2) and ascorbic acid (T_{10}) along with KMS packed in LDPE bags and stored in refrigerated temperature (0.980). Lower PPO activity was

noticed in all fruit pulp pre-treated with ascorbic acid after one month of storage, fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in LDPE bags and stored in refrigerated temperature (T_{14}) after two months and fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature (T_{10}) after 3 months of storage. Microbial population increased during storage and comparatively high counts were seen after second and third months of storage, fruit pulp pre-treated with ascorbic acid and sodium benzoate packed in glass jars and stored in refrigerated temperature (T_{16}) (nil) and fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature (T_{10}) recorded the lower microbial population. All the samples stored under ambient conditions became unmarketable within one month of storage, irrespective of the pre-treatments. Two months after storage, refrigerated samples had higher sensorial acceptability in appearance, colour and texture while flavour, odour, taste and after taste had lower scores and after 3 months of storage only fruit pulp pre-treated with citric acid and KMS packed in glass jars and stored in refrigerated temperature (T_4), fruit pulp pre-treated with ascorbic acid and KMS packed in LDPE bags and stored in refrigerated temperature (T_{10}) and fruit pulp pre-treated with ascorbic acid and KMS packed in glass jars and stored in refrigerated temperature (T_{12}) obtained acceptable scores for appearance and colour.

Considering the longer shelf life, significantly higher total protein, total carbohydrate, total fat and organoleptic scores and lower water activity, polyphenoloxidase activity, and microbial population, avocado pulp added with ascorbic acid along with KMS followed by packging in LDPE bags and stored under refrigeration (T_{10}) and also the pulp with same additives packed in glass jars stored under refrigeration (T_{12}) were selected as an ideal pre-treatment methods for the preservation of avocado pulp.

In the experiment IVb, optimization of process conditions for preparation of avocado fruit powder was carried out by adding the fruit pulp with additives such as maltodextrin (2, 3, 4 and 5 %), ascorbic acid (1 %), tricalcium phosphate (0.15 %), EDTA (0.05 %) and potassium sorbate (0.05 %) followed by freeze drying. The obtained powder was packed in LDPE laminated Aluminium pouches (200 gauge) and in glass jars and subsequently stored under ambient and refrigerated conditions for 3 months.

During storage bulk density of the powder decreased during storage. Higher values were seen in pulp added with 5% maltodextrin (T_{16}) after 1 month (0.45 g/cm^3), and pulp added with 4% (T_{10}) (0.27 g/cm^3) and 5 % (T_{14}) (0.26 g/cm^3) maltodextrin after 2 months storage. and those with 2% (T_2) and 5% (T_{14}) maltodextrin packed in LDPE bags and stored in refrigeration (0.23 g/cm^3) after 3 months. Solubility decreased during storage and significantly higher solubility were seen in fruit pulp pre-treated with higher concentration of maltodextrin. Hygroscopicity also increased during storage period of three months and the lowest values were seen in fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature (T_{16}) after first (0.25 %) and second (0.33 %) months and in fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature (T_{14}) (1.31 %) after third month of storage. Higher colour value L* was noticed in fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in ambient temperature (T_{12}) (76.64), after one month and in fruit pulp pre-treated with 4 % maltodextrin packed in LDPE bags and stored in refrigerated temperature (T_{10}) (53.28) after two months and fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in ambient temperature (T_{12}) (42.60) after three months of storage. Higher L* value were seen in fruit powder with higher maltodextrin content. Hunter colour value a* increased up to three months of storage indicated the decrease of green colour and higher negative values were seen in fruit pulp pre-treated with 5 % maltodextrin packed in both LDPE bags (T_{14}) and glass jars (T_{16}), stored in refrigerated temperature after first (-14.21 and -14.18) and second (-3.63 and -3.63) months, respectively and in fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in ambient temperature (T_{12}) (-0.38) after three months of storage. Colour value b* also increased during storage, indicated the increase of yellow colour and the lower values were observed in fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature (T_{14}) after one (25.30) and 2 (40.00) months of storage and fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature (T_{16}) (86.46) after three months of storage. Higher TSS content was observed in fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags (T_{14}) (4.13 ^{o}Brix) and glass jars (T_{16}) (7.70 ^{o}Brix) stored in refrigerated temperature after one and two months of storage. Titratable acidity varied non-significantly throughout the storage and the total protein content decreased during storage with significantly higher values in fruit pulp pre-treated with 3 % maltodextrin

packed in glass jars and stored in refrigerated temperature (T_8) (6.24 g/100g) and fruit pulp pre-treated with 4 % maltodextrin packed in glass jars and stored in ambient temperature (T_{12}) (0.91 g/100g) after 2 and 3 months of storage. A rise in vitamin C content was seen after 1 month of storage and fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature (T_{14}) (70 mg/100g) and in fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in refrigerated temperature (T_8) (13.33 mg/100g) had significantly higher value after one and three months of storage at 0.05 % level. Total phenols increased during storage and varied non-significanlty among treatments. Total carbohydrates decreased after one month of storage and significantly higher values were observed in fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags (T_{14}) (35.20 g/100g) and glass jars (T_{16}) (34.80 g/100g) stored in refrigerated temperature after two months of storage. Total fat decreased throughout storage and significantly higher values were observed in fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags (T_{14}) (60.43 %) and glass jars (T_{16}) (61.20 %) stored in refrigerated temperature after one month and two months of storage. Peroxide value increased after two months of storage and lower values were found in samples pre-treated with 4 and 5 % maltodextrin after one and three months of storage at 0.05 % level of significance. Water activity increased with significantly lower values in fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature (T_{14}) and fruit pulp pre-treated with 5 % maltodextrin packed in glass jars and stored in refrigerated temperature (T_{16}) throughout storage. Microbial colonies remained non-significant during storage among treatments and were observed only after two months of storage in which fungal colonies were nil in fruit pulp pre-treated with 3 % maltodextrin packed in glass jars and stored in refrigerated temperature (T_8) and fruit pulp pre-treated with 5 % maltodextrin packed in LDPE bags and stored in refrigerated temperature (T_{14}) and lower bacterial colonies (1.17 cfu x 10^5/g) were observed in T_{10}. After 3 months of storage, lower count of fungal and bacterial colonies were observed in the same treatments with 0.33 cfu x 10^3/g and 1.67 cfu x 10^5/g, respectively. During storage, organoleptic qualities of all the samples decreased and parameters such as appearance, colour and texture obtained comparatively higher scores than odour, flavour, taste and after taste. By using the freeze dried avocado powder, preparation of an instant fruit shake was standardised by blending the fruit powder, skimmed milk powder, sucrose and water in the proportion of 1:2:1:2 with better sensorial acceptability.

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APPENDICES

APPENDIX I

Media composition

1. NUTRIENT AGAR MEDIA (FOR BACTERIA)

Beef extract	: 3 g
Peptone	: 5 g
Sodium chloride	: 5 g
Agar	: 18g
Distilled water	: 1000ml
pH	: 6.8-7.2

2. ROSE BENGAL AGAR MEDIA (FOR FUNGUS)

Papaic digest of soyabean meal	: 5 g
Dextrose	: 10 g
Monopotassium phosphate	: 1 g
Magnesium sulphate	: 0.50 g
Rose Bengal	: 0.05g
Agar	: 15 g
pH	: 5.6

3. SABAURAUD DEXTROSE AGAR (FOR YEAST)

Mycological peptone	: 10 g
Dextrose	: 40 g
Agar	: 15 g
Distilled water	: 1000 ml
pH	: 5.6

APPENDIX II

Score card for organoleptic evaluation of green chilies

Name of the judge:

Date:

Characteristics	Score				
	T_0	T_1	T_2	T_3	T_4
Appearance					
Colour					
Texture					
Odour					
Overall acceptability					

9 point Hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Signature

POST HARVEST CHARACTERISATION AND MANAGEMENT OF AVOCADO (*Persea americana* Mill.)

By

GEETHU M.

(2018-22-006)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN HORTICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF POST HARVEST TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR -680 656

KERALA, INDIA

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ABSTRACT

Avocado is a subtropical fruit crop, belonging to the family Lauraceae, and is rich in proteins, vitamins, minerals and monounsaturated fatty acids such as oleic acid, contributing to its high nutritive and therapeutic value. Even though there are a large number of genotypes with widely varying characteristics, inadequate characterisation and identification result in the lack of awareness, improper utilisation and insufficient post harvest management of avocado. Hence, the present study titled, 'Post harvest characterisation and management of avocado (*Persea americana* Mill.)' was carried out in the Department of Post Harvest Technology, College of Agriculture, Vellanikkara during 2018-2021. The main objectives of the study were to characterise avocado accessions collected from different parts of Kerala and to evaluate the effect of post harvest management practices to extend the shelf life of avocado fruits and to study the effect of food additives on the quality of frozen slices, fruit pulp, freeze dried fruit powder and subsequently to standardise an instant avocado fruit shake.

For the characterisation of avocado genotypes, 27 accessions were collected, among which 14 accessions were from RARS, Ambalavayal in Wayanad and 12 from Kanthaloor in Idukki and one accession was collected from Thanniyam in Thrissur. Characterisation of avocado genotypes based on the horticultural and biochemical traits, accession 25 from Idukki had comparatively higher TSS, vitamin C, total carbohydrates, total flavonoids, oleic acid, calcium, potassium, iron, total ash and crude fibre content. Hence, accession 25 was selected for subsequent post harvest management studies. Antioxidant activity of the methanolic extract of fresh fruit of accession 25 was evaluated by DPPH, FRAP and ABTS assays. Greatest free radical scavenging activity was observed in ABTS assay with lowest IC_{50} value of 0.10 µg/mL.

Fresh and mature avocado fruits, surface sanitised with 2 ppm ozone and pre-treated with 2 % calcium chloride, followed by shrink packaging with 25 µ polyolefin film and subsequently stored in refrigeration (T_8) as well as cool chamber (T_9) were found to be the ideal storage conditions with longest shelf life of 27 days. Calcium chloride pre-treated fruits with shrink packaging, stored under refrigeration had lowest physiological loss in weight, respiration rate, ethylene evolution rate and decay per cent, with better retention of firmness. Fruits of this treatment retained significantly higher total carbohydrates, total protein and total phenols during storage.

Avocado slices pre-treated with 40 % sucrose, ascorbic acid (0.5 %) and potassium metabisulphite (0.1 %), quick frozen to -20 ºCin 30 minutes followed by packing in 200 gauge LDPE pouches and held under frozen temperature (-18 ^{0}C) was the most ideal pre-treatment for storage. This treatment recorded significantly higher TSS, vitamin C, total carbohydrates, total protein and organoleptic acceptability throughout storage and lowest water activity, peroxide value and microbial population.

For preparation and storage of avocado pulp, pre-treatments with ascorbic acid (0.5%) and KMS (0.1%) followed by vacuum packaging LDPE bags (T_{10}) as well as in glass jars (T_{12}), stored under refrigeration resulted in longest shelf life and better quality. Total protein, total phenols, total carbohydrate, total fat, viscosity and organoleptic scores were highest in these treatments with lowest water activity, polyphenol oxidase activity and microbial population during storage.

For preparation of avocado fruit powder, addition of 5% maltodextrin, ascorbic acid (1%), tricalcium phosphate (0.15%), EDTA (0.05%) and potassium sorbate (0.05%) followed by freeze drying at -70 ºCand 100 mtorr vacuum for 36 hours followed by packing in LDPE laminated aluminium pouches (T_{14}) and glass jars (T_{16}) stored under refrigeration were the ideal methods with longest shelf life and quality. Significantly higher bulk density, solubility, colour value L*, TSS, vitamin C, total carbohydrates, total fat and organoleptic scores were recorded in these treatments during storage along with lowest hygroscopicity, colour value a* and b*, peroxide value, water activity and microbial population.

An instant avocado shake was standardised by combining avocado fruit powder with skimmed milk powder, sucrose and water in the proportion of 1:2:1:2 with appealing appearance, light yellowish colour, unique blend of taste and flavour of avocado fruit and skimmed milk powder.

സംക്ഷിപ്തം

ലോറേസി കുടുംബത്തിൽ പെടുന്ന ഒരു ഉപ ഉഷ്ണമേഖലാ ഫലവിളയാണ് അവോക്കാഡോ. പ്രോട്ടീനുകൾ, വിറ്റാമിനുകൾ, ധാതുക്കൾ. മോണോസാച്ചുറേറ്റഡ് ഫാറ്റി ആസിഡുകളായ ഒലിക് ആസിഡ് എന്നിവയാൽ സമ്പന്നമാണ്, ഇത് ഉയർന്ന പോഷകമൂല്യവും ചികിത്സാ മൂല്യവും നൽകുന്നു. വൈവിധ്യമാർന്ന സ്വഭാവസവിശേഷതകളുള്ള ധാരാളം ജനിതകരൂപങ്ങൾ ഉണ്ടെങ്കിലും, അവോക്കാഡോയെക്കുറിച്ച് ഉള്ള അവബോധമില്ലായ്മ, അപര്യാപ്തമായ തിരിച്ചറിയലും സ്വഭാവരൂപീകരണവും, അനുചിതമായ വിനിയോഗം, വിളവെടുപ്പിനു ശേഷമുള്ള അപര്യാപ്തമായ പരിപാലനം എന്നിവയ്ക്ക് കാരണമാകുന്നു. അതിനാൽ, 2018-2021 കാലയളവിൽ വെള്ളാനിക്കരയിലെ കാർഷിക കോളേജിലെ പോസ്റ്റ് ഹാർവെസ്റ്റ് ടെക്നോളജി ഡിപ്പാർട്ട്മെന്റിൽ, 'അവക്കാഡോയുടെ വിളവെടുപ്പിന് ശേഷമുള്ള സ്വഭാവവും പരിപാലനവും' എന്ന തലക്കെട്ടിലുള്ള ഇപ്പോഴത്തെ പഠനം നടത്തി. കേരളത്തിന്റെ വിവിധ ഭാഗങ്ങളിൽ നിന്ന് ശേഖരിക്കുന്ന അവോക്കാഡോകളുടെ സ്വഭാവരൂപീകരണവും അവോക്കാഡോ പഴങ്ങളുടെ ആയുസ്സ് വർദ്ധിപ്പിക്കുന്നതിനുള്ള വിളവെടുപ്പിനു ശേഷമുള്ള പരിപാലന രീതികളുടെ ഫലത്തെ വിലയിരുത്തുകയും ചെയ്യുക, ശീതീകരിച്ച കഷ്ണങ്ങളുടെ ഗുണനിലവാരത്തിൽ ഭക്ഷ്യ അഡിറ്റീവുകളുടെ സ്വാധീനം പഠിക്കുക, ഫ്രൂട്ട് പൾപ്പ്, ഫ്രീസ് ഡ്രൈ ഫ്രൂട്ട് പൗഡർ, തുടർന്ന് ഒരു തൽക്ഷണ അവോക്കാഡോ ഫ്രൂട്ട് ഷേക്ക് സ്റ്റാൻഡേർഡൈസ് ചെയ്യുക എന്നതായിരുന്നു പഠനത്തിന്റെ പ്രധാന ലക്ഷ്യങ്ങൾ.

അവോക്കാഡോ ജനിതകരൂപങ്ങളുടെ സ്വഭാവരൂപീകരണത്തിനായി, 27 തരം ഇനങ്ങൾ ശേഖരിച്ചു, അതിൽ 14 ഇനങ്ങൾ ആർഎആർഎസ്, അമ്പലവയൽ, വയനാട്, 12 എണ്ണം ഇടുക്കിയിലെ കാന്തലൂർ എന്നിവിടങ്ങളിൽ നിന്നും തൃശ്ശൂരിലെ താന്നിയത്ത് നിന്ന് ഒരു ഇനവും ശേഖരിച്ചു. ഹോർട്ടികൾച്ചറൽ, ബയോകെമിക്കൽ സ്വഭാവങ്ങളെ അടിസ്ഥാനമാക്കിയുള്ള അവോക്കാഡോ ജനിതകരൂപങ്ങളുടെ സ്വഭാവം, ഇടുക്കിയിൽ നിന്നുള്ള ഇനം 25-ൽ താരതമ്യേന ഉയർന്ന TSS, വിറ്റാമിൻ സി, മൊത്തം കാർബോഹൈഡ്രേറ്റ്, മൊത്തം ഫ്ലേവനോയ്ഡുകൾ, ഒലിക് ആസിഡ്, കാൽസ്യം, പൊട്ടാസ്യം, ഇരുമ്പ്, മൊത്തം ചാരം, ക്രൂഡ് ഫൈബർ എന്നിവ ഉണ്ടായിരുന്നു.

അതിനാൽ, തുടർന്നുള്ള വിളവെടുപ്പിനു ശേഷമുള്ള പരിപാലനപഠനത്തിനായി ഇനം 25 തിരഞ്ഞെടുത്തു. അക്സഷൻ 25-ന്റെ പുതിയ പഴത്തിന്റെ മെത്തനോളിക് സത്തിൽ ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം ഡിപിപിഎച്ച്, എഫ്ആർഎപി, എബിടിഎസ് എന്നിവ വിലയിരുത്തി. ഏറ്റവും കുറഞ്ഞ ഐ സി 50 മൂല്യമായ 0.10 µg/mL ഉള്ള എബിടിഎസ് പരിശോധനയിൽ ഏറ്റവും മികച്ച സ്വതന്ത്ര റാഡിക്കലുകൾ നീക്കം ചെയ്യാനുള്ള കഴിവ് നിരീക്ഷിക്കപ്പെട്ടു.

പുതിയതും മുതിർന്നതുമായ അവോക്കാഡോ പഴങ്ങൾ, ഉപരിതലത്തിൽ 2 ppm ഓസോൺ ഉപയോഗിച്ച് അണുവിമുക്തമാക്കുകയും 2% കാൽസ്യം ക്ലോറൈഡ് ഉപയോഗിച്ച് പ്രീ-ട്രീറ്റ് ചെയ്യുകയും, തുടർന്ന് 25 µ പോളിയോലിഫിൻ ഫിലിം ഉപയോഗിച്ച് ചുരുക്കി പാക്കേജിംഗ് ചെയ്യുകയും തുടർന്ന് റഫ്രിജറേഷനിലും കൂൾ ചേമ്പറിലും സൂക്ഷിക്കുകയും ചെയ്തു. അത് 27 ദിവസത്തെ ഏറ്റവും ദൈർഘ്യമേറിയ ഷെൽഫ് ലൈഫ് ഉള്ള അനുയോജ്യമായ സംഭരണ അവസ്ഥയാണെന്ന് കണ്ടെത്തി. കാത്സ്യം ക്ലോറൈഡ് മുൻകൂട്ടി സംസ്കരിച്ച പഴങ്ങൾ; ഫ്രിഡ്ജിൽ സൂക്ഷിച്ചിരിക്കുന്ന ചുരുക്കൽ പാക്കേജിംഗിൽ, ഭാരം, ശ്വസന നിരക്ക്, എഥിലീൻ വാതകം പുറത്തിറക്കുന്ന നിരക്ക്, ശോഷണം ശതമാനം എന്നിവയിൽ ഏറ്റവും കുറഞ്ഞ ശാരീരിക നഷ്ടം, കൂടാതെ മികച്ച ദൃഢത നിലനിർത്തുന്നു. ഈ ചികിത്സയുടെ പഴങ്ങൾ സംഭരണ സമയത്ത് ഗണ്യമായി ഉയർന്ന മൊത്തം കാർബോഹൈഡ്രേറ്റ്, മൊത്തം പ്രോട്ടീൻ, മൊത്തം ഫിനോൾ എന്നിവ നിലനിർത്തി.

അവോക്കാഡോ കഷ്ണങ്ങൾ 40% സുക്രോസ്, അസ്കോർബിക് ആസിഡ് (0.5%), പൊട്ടാസ്യം മെറ്റാബിസൾഫൈറ്റ് (0.1%) എന്നിവ ഉപയോഗിച്ച് പ്രീ-ട്രീറ്റ് ചെയ്തു, പെട്ടെന്ന് ഫ്രീസുചെയ്ത് -20 °C ൽ 30 മിനിറ്റ്, തുടർന്ന് 200 ഗേജ് എൽഡിപിഇ പൗച്ചുകളിൽ പാക്ക് ചെയ്ത് ശീതീകരിച്ച താപനില (-18 °C) സംഭരണത്തിനുള്ള ഏറ്റവും അനുയോജ്യമായ പ്രീ-ട്രീറ്റ്മെന്റായിരുന്നു. ഈ സംഭരണത്തിൽ ഗണ്യമായി ഉയർന്ന ടിഎസ്എസ്, വിറ്റാമിൻ സി, മൊത്തം കാർബോഹൈഡ്രേറ്റ്, മൊത്തം പ്രോട്ടീൻ, ഓർഗാനോലെപ്റ്റിക് സ്വീകാര്യത, എന്നിവയെല്ലാം കൂടാതെ കുറഞ്ഞ ജല പ്രവർത്തനത്തിലും പെറോക്സൈഡ് മൂല്യവും സൂക്ഷ്മജീവികളുടെ ജനസംഖ്യയും രേഖപ്പെടുത്തി.

അവോക്കാഡോ പൾപ്പ് തയ്യാറാക്കുന്നതിനും സംഭരിക്കുന്നതിനും, അസ്കോർബിക് ആസിഡ് (0.5%), കെഎംഎസ് (0.1%) എന്നിവയ്ക്ക് ശേഷം വാക്വം പാക്കേജിംഗ് എൽഡിപിഇ ബാഗുകളും ഗ്ലാസ് ജാറുകളിലും ശീതീകരണത്തിൽ സൂക്ഷിച്ചിരിക്കുന്ന പ്രീ-ട്രീറ്റ്മെന്റുകൾ ഏറ്റവും നീളമുള്ള സംഭരണശേഷിയും, മികച്ച നിലവാരവും കാണിച്ചിരുന്നു. മൊത്തം പ്രോട്ടീൻ, മൊത്തം ഫിനോൾ, മൊത്തം കാർബോഹൈഡ്രേറ്റ്, മൊത്തം കൊഴുപ്പ്, വിസ്കോസിറ്റി, ഓർഗാനോലെപ്റ്റിക് സ്കോറുകൾ ഒപ്പം ഏറ്റവും കുറഞ്ഞ ജല പ്രവർത്തനവും പോളിഫെനോൾ ഓക്സിഡേസ് പ്രവർത്തനവും സംഭരണ സമയത്ത് സൂക്ഷ്മജീവികളുടെ ജനസംഖ്യയും ഉള്ള ഈ ചികിത്സ മികച്ചതായിരുന്നു.

അവോക്കാഡോ ഫ്രൂട്ട് പൗഡർ തയ്യാറാക്കുന്നതിനായി, 5% മാൾട്ടോഡെക്സ്ട്രിൻ, അസ്കോർബിക് ആസിഡ് (1%), ട്രിക്കൽസിയം ഫോസ്ഫേറ്റ് (0.15%), ഇഡിടിഎ (0.05%), പൊട്ടാസ്യം സോർബേറ്റ് (0.05%) എന്നിവ ചേർത്ത് -70 °C ഒപ്പം 100 mtorr ശൂന്യതയിലും 36 മണിക്കൂർ നേരം മരവിപ്പിച്ചു ഉണക്കി, LDPE ലാമിനേറ്റഡ് അലുമിനിയം പൗച്ചുകളിൽ (T14), ഗ്ലാസ് ജാറുകൾ (T16) എന്നിവയിൽ പാക്ക് ചെയ്യുക ശീതീകരണത്തിൽ സൂക്ഷിച്ചിരിക്കുക എന്നതു പൊടിയുടെ ദൈർഘ്യമേറിയ ആയുസ്സും ഗുണമേന്മയുമുള്ള അനുയോജ്യമായ മാർഗ്ഗങ്ങളായിരുന്നു. ഗണ്യമായി ഉയർന്ന ബൾക്ക് ഡെൻസിറ്റി, സോളബിലിറ്റി, കളർ മൂല്യം എൽ*, ടിഎസ്എസ്, വിറ്റാമിൻ സി, മൊത്തം കാർബോഹൈഡ്രേറ്റ്സ്, മൊത്തം കൊഴുപ്പ്, ഓർഗാനോലെപ്റ്റിക് സ്കോറുകൾ എന്നിവ ഈ ചികിത്സകളിൽ രേഖപ്പെടുത്തിയിട്ടുണ്ട്, കൂടാതെ ഏറ്റവും കുറഞ്ഞ ഹൈഗ്രോസ്കോപ്പിസിറ്റി, കളർ മൂല്യം a*, b*, പെറോക്സൈഡ് മൂല്യം, ജല പ്രവർത്തനം, സൂക്ഷ്മജീവികളുടെ ജനസംഖ്യയും.

1:2:1:2 എന്ന അനുപാതത്തിൽ അവോക്കാഡോ ഫ്രൂട്ട് പൗഡറും സ്കിംഡ് മിൽക്ക് പൗഡറും സുക്രോസും വെള്ളവും ചേർത്ത് ആകർഷകമായ രൂപവും ഇളം മഞ്ഞകലർന്ന നിറവും അവോക്കാഡോ പഴത്തിന്റെയും സ്കിംഡ് പാലിന്റെയും സവിശേഷമായ രുചിയും സ്വാദും കൊണ്ട് ഒരു തൽക്ഷണ അവോക്കാഡോ ഷേക്ക് സ്റ്റാൻഡേർഡ് ചെയ്തു.