



# Physico-chemical, Sensorial and Microbiological Quality of Frozen Avocado (*Persea americana* Mill.) Slices

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## ABSTRACT

**Background:** Avocado (*Persea americana* Mill), is a subtropical, evergreen fruit tree belonging to the family Lauraceae. Due to the unique climacteric nature of the fruit, avocado starts ripening immediately after harvest and have poor post harvest life. Since thermal processing has adverse effects on the fruit quality, avocado processing is challenging.

**Methods:** Avocado fruit slices were subjected to quick freezing (-20°C within 30 minutes) after pre-treatment with sucrose (20-40%), ascorbic acid (0.5%) along with one of the antimicrobial compounds like potassium metabisulphite, sodium benzoate or potassium sorbate (0.1%) and subsequently packed in 200 gauge LDPE pouches stored at -18°C.

**Result:** Avocado slices treated with 40% sucrose, 0.5% ascorbic acid along with 0.1% potassium metabisulphite (T<sub>7</sub>) recorded with higher ascorbic acid (92 mg/100 g) after one month and total protein (1.43 g/100 g) content after three months of storage and desirably lowest values in polyphenol oxidase activity throughout the storage, peroxide value (15.56 mEq/kg) and water activity (0.956) after one month of storage was observed as the best method of preservation.

**Key words:** Avocado, Microbial, Physico-chemical, Quick freezing, Sensory.

## INTRODUCTION

Avocado (*Persea americana* Mill), is a subtropical, evergreen fruit tree belonging to the family Lauraceae. Avocado is the most nutritive among fruits with highest energy value of 245 cal/100 g. The fruit pulp is rich in proteins (4%) and fat (30%), but low in carbohydrates (5.1 g/100 g). Avocado fruit is a reservoir of several vitamins such as A, B, C and E and minerals like potassium, calcium and phosphorous. Avocado fat composition is similar to olive oil, rich in mono-unsaturated fatty acids which reduce 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. As it is a seasonal fruit crop, lack of appropriate processing and storage conditions results in huge losses. 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 the application of quick freezing, along with pretreatment of acidulants such as ascorbic acid (Pauker, 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).

As conventional avocado processing have many detrimental effects, there is scarcity of studies regarding the processing and preservation of avocado. Thus, the present investigation explores the suitability of pre-treatments of quick frozen avocado slices.

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## MATERIALS AND METHODS

The study was conducted in the department of Post Harvest Technology, Kerala Agricultural University, in the year 2018-2021. Avocado fruits of a local accession collected from Kanthaloor, Idukki district of Kerala, located at 1525m above MSL were used for the study. Mature ripe fruits were surface sanitised in ozonised water using ozonizer (Make: Mine-Q Anion, India) at 2 ppm concentration. The flesh obtained after removal of peel and seed was cut into longitudinal slices of 1.5-2 cm thickness. Avocado slices were immersed in a solution with different concentrations of sucrose at 20, 30 and 40%, in combination with 0.5% ascorbic acid along with one of the preservatives like potassium metabisulphite, sodium benzoate or potassium sorbate at 0.1% concentration. The pre-treated fruit slices were quick frozen to -20°C within 30 minutes using a blast freezer (Make: Celfrost D3, Italy), wherein cold air was blown across the fruit slices at a velocity of 2.3 to 2.8 m/sec. The frozen fruit

slices were packed in LDPE (200 gauge) pouches and were subsequently stored at  $-18^{\circ}\text{C}$  for three months. The whole experiment consisted of nine treatments in which avocado slices were added with ascorbic acid and additives such as 20% sucrose + potassium metabisulphite ( $T_1$ ), 20% sucrose + sodium benzoate ( $T_2$ ), 20% sucrose + potassium sorbate ( $T_3$ ), 30% sucrose + potassium metabisulphite ( $T_4$ ), 30% sucrose + sodium benzoate ( $T_5$ ), 30% sucrose + potassium sorbate ( $T_6$ ), 40% sucrose + potassium metabisulphite ( $T_7$ ), 40% sucrose + sodium benzoate ( $T_8$ ), 40% sucrose + potassium sorbate ( $T_9$ ).

There were three replications in each treatment and each replication consisted of 10 samples of about 250 g slices. All the biochemical parameters were analysed using completely randomised design (CRD) and organoleptic evaluation was analysed using Kendall's coefficient of concordance. The changes in quality parameters were observed at monthly intervals.

Total soluble solids (TSS) was measured using a digital refractometer (Make: ATAGO PAL-1, 0-53 $^{\circ}$ Brix, Japan) and expressed in  $^{\circ}$ Brix. Titrable acidity was measured according to AOAC (1998) and expressed in per cent of malic acid which is the predominant acid in avocado. Vitamin C content was determined according to AOAC (1998). Total phenol content was measured using Folin-Ciocalteu reagent and expressed as milligram per 100 g (Asami *et al.* 2003). Total carbohydrate content was determined using anthrone reagent and expressed in gram per 100 g (Hewitt, 1958). Total protein was measured according to Lowry *et al.* (1951). at 660 nm and expressed in g/100 g. Total fat was determined from the dry weight of sample extracted in soxhlet apparatus using petroleum ether (Ranganna, 1986). Polyphenol oxidase activity was determined according to Fujita *et al.* (1995) using 100 mM sodium phosphate buffer solution and expressed in unit absorbance/min.  $\text{mL}^{-1}$  of sample. Peroxide values were calculated according to AOCS (1998) using potassium iodide and 20 mL of solvent mixture (2:1 mixture of glacial acetic acid and chloroform), titrated against N/500 sodium thiosulphate solution. Water activity ( $a_w$ ) of the samples were measured using a water activity meter (Make: AquaLab, Pre 40412, Decagon Devices, USA) at 25 to  $28^{\circ}\text{C}$ . Quantitative assay of microbial load was conducted using serial dilution spread plate technique suggested by Agarwal

and Hasija (1986) and expressed as  $\text{cfu}10\text{g}^{-1}$  of sample. Organoleptic evaluation was conducted using 9 point hedonic scale for sensory attributes viz. appearance, colour, odour, flavour, texture, taste, after taste and overall acceptability by a semi trained panel of 15 members at monthly intervals (Peryam and Pilgrim, 1957).

## RESULTS AND DISCUSSION

### Total soluble solids

TSS of the fruit immediately after pre-treatment and just before frozen storage was 6.33  $^{\circ}$ Brix (Fig 1). It increased up to one month and thereafter decreased throughout the remaining storage period without much difference. Significantly higher TSS were recorded in the fruit slices pre-treated with 40% sucrose throughout the storage which may be due to the lowering of free water availability and concentration of soluble solids under frozen storage ( $-18^{\circ}\text{C}$ ). Fruit slices pre-treated with  $T_7$  had the highest TSS (26 $^{\circ}$ Brix) and lowest (17 $^{\circ}$ Brix) in  $T_5$ , after one month of storage. 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 $^{\circ}$ Brix. Inversion of polysaccharides and added sucrose into simple soluble substances during storage might increase TSS (Smitha and Sreeramu, 2019).

### Titrateable acidity (%)

Initial titrateable acidity of the avocado fruit was 1.34% (Fig 1). It decreased during storage with proportionate increase in the concentration of sucrose. Significantly highest (0.56%) and lowest titrateable acidity (0.22%) were seen in  $T_1$  and  $T_9$  at only two months after storage which may be attributed to the increase in soluble solids under frozen storage. Nardos and Wakgari, (2016) mentioned that higher concentration of sucrose and storage under low temperature increased sugar content and decreased titrateable acidity in the avocado fruits slices.

### Vitamin C (mg/100 g)

Initial vitamin C content of avocado fruit was 14.67 mg/100 g (Fig 1) which increased in the initial phase of storage and decreased towards the end. Initial increase in vitamin C was due to pre-treatment of fruit slices with ascorbic acid.

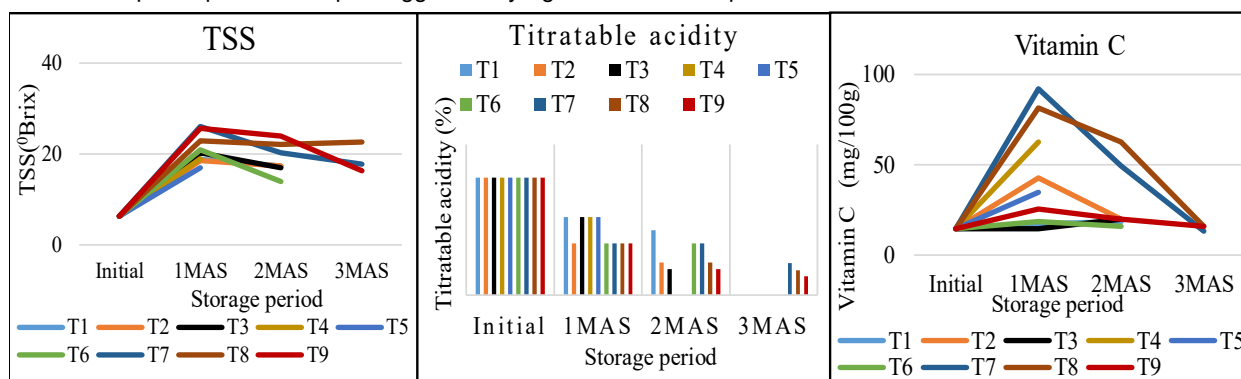


Fig 1: TSS, titrateable acidity and vitamin C content of frozen avocado slices during storage.

Significantly highest value was recorded in samples treated with T<sub>7</sub> (92.00 mg/100 g) after one month of storage. Lower levels of vitamin C were noticed in fruit slices treated with potassium sorbate at different concentrations of sucrose. De Ancos *et al.* (2000) reported that during freezing cell damages to the fruit resulted in the release of vitamin C in raspberries. 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.

#### Total carbohydrate (g/100g)

Avocado had an initial total carbohydrate content of 9.87 g/100g (Fig 2) decreased in frozen avocado slices during storage. Fruit slices treated with T<sub>7</sub> had significantly higher total carbohydrate (8.83 g/100 g) and lowest (3.37 g/100 g) in T<sub>6</sub> after 1 month of storage. Higher total carbohydrate content in the treatment T<sub>7</sub> may be due to the higher sucrose concentration used as pre-treatment. Initial increase in carbohydrate may be the result of intake of solids and release of water from fruit samples as ice crystals in osmotic solution under frozen storage (Sikora *et al.* 2013). Decrease in total carbohydrate during storage may be due to the metabolic activity as stored sugars contribute to carbon energy source for respiratory process during postharvest storage (Liu *et al.* 1999).

#### Total phenols (mg/100 g)

Avocado fruit had an initial total phenols content of 36.5

mg/100 g (Fig 2). Total phenols increased initially during storage which may be due to the rupture of fruit tissue resulted in release of phenolic compounds during preparation of frozen avocados, followed by a decrease towards the end of storage. At 1 and 2 months after storage significantly higher phenols were seen in fruit slices treated with T<sub>4</sub> (96.67 mg/100 g) and T<sub>6</sub> (98.30 mg/100 g) and lower values in T<sub>7</sub> (56.67 mg/100 g) and T<sub>9</sub> (76.67 mg/100 g). 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.

#### Total protein (g/100 g)

Fresh avocado fruits had a total protein content of 0.875 g/100g (Fig 2) and decreased during storage followed by an increase towards the end of storage in fruit slices pretreated with 40% sucrose. Significantly higher protein contents (0.857, 0.782 and 1.43 g/100 g) were found in fruit slices treated with T<sub>7</sub> after 1, 2 and 3 months of storage, respectively. Lower total protein content was observed in T<sub>4</sub> (0.408 g/100 g) and T<sub>5</sub> (0.437 g/100 g) which had shorter shelf life due to faster deterioration with loss of texture. Reduction in total protein content of fruit slices may be due to its utilisation for metabolic activities. Heat shock proteins protect and retain other proteins and enzyme systems during heat treatment or cold storage (Hofman *et al.* 2002). Sikora *et al.* (2013)

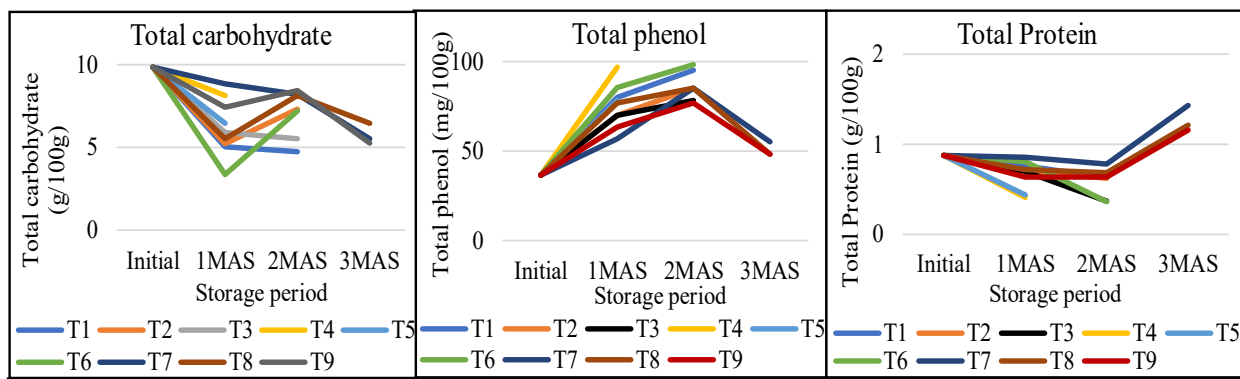


Fig 2: Total carbohydrate, total phenol and total protein content of frozen avocado slices during storage.

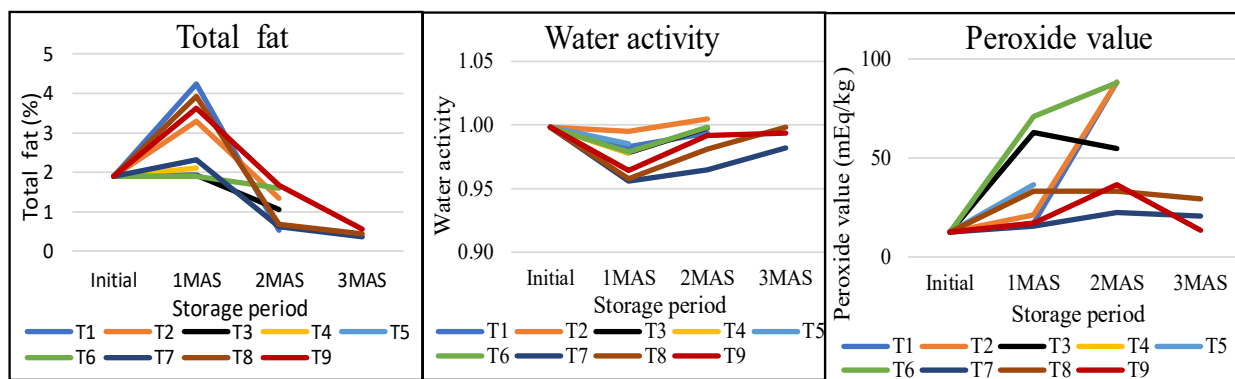


Fig 3: Total fat content, water activity and peroxide value of frozen avocado slices during storage.

mentioned that change in proteins of fruits under frozen storage conditions were non-significant in blackthorn fruits.

#### Total fat (%)

Avocado had an initial fat content of 1.9% (Fig 3) and increased after one month of storage and thereafter, decreased throughout the remaining storage period. Total fat content did not vary significantly in any of the samples. However,  $T_1$  had the highest fat content (4.25%) and  $T_6$  had the lowest (1.90%) after one month of storage. Initial increase in fat content may be due to the leaching out of water from the sample immersed in osmotic solution and further decrease may be due to the oxidative deterioration. It was confirmed in the findings of Mepba *et al.* (2008) in avocado paste. The cessation in the amount of accumulated sugars and TSS correlate with the accumulation of oil in fruits during postharvest storage (Liu *et al.* 1999).

#### Peroxide value

Avocado pulp had an initial peroxide value of 12.64 mEq/kg (Fig 3). After one month of storage, highest peroxide value (71.11 mEq/kg) was reported in  $T_6$ . The least peroxide value was observed in  $T_7$  (15.56 mEq/kg), which is an indication of reduction in oxidation and subsequent rancidity and retention of flavour. Significantly lower peroxide values were obtained in all the samples preserved with potassium metabisulphite. The oxidative deterioration of avocado slices was marginal except the rancid taste towards the end of storage. The activity of peroxidase enzyme decreased with the maturation of the fruit and increased with temperature and time (Murasaki, 2009).

#### Polyphenol oxidase activity (PPO)

PPO activity increased with increase in time and the lowest enzyme activity was observed in fruit slices pre-treated with  $T_7$  after 1, 2 and 3 months of storage (Fig 4). With a residual PPO activity <45% and storage at 5°C maintained an acceptable colour in avocado puree for at least 60 days (López-Malo *et al.* 1998).

#### Water activity

Water activity of the fresh avocado fruit sample was 0.998. A decrease in water activity was noticed in the initial phase of storage and thereafter it increased till the end of storage (Fig 3). The initial decrease in water activity may be due to the osmotic effect on the fruit slices. After one month of storage, lowest water activity (0.956) was noticed in  $T_7$  and highest water activity (0.995) was noticed in  $T_2$ . Giannakourou *et al.* (2020) reported that osmotic pre-treatment with the application of sucrose decreased product water activity by the mass transfer phenomenon and reduced the freezing point accomplished by water loss and solid impregnation.

#### Microbial population

Pre-treatments to avocado slices before freezing were done with the intention of consuming it uncooked, therefore microbial count is a major concern. It was observed that the microbial population increased with the storage time, where

**Table 1:** Microbial population of frozen avocado slices during storage (cfu/10 g).

	Initial			1 MAS			2 MAS			3 MAS		
	Bacteria (10 <sup>5</sup> cfu/10g)	Yeast (10 <sup>4</sup> cfu/10g)	Fungi (10 <sup>3</sup> cfu/10g)	Bacteria (10 <sup>5</sup> cfu/10g)	Yeast (10 <sup>4</sup> cfu/10g)	Fungi (10 <sup>3</sup> cfu/10g)	Bacteria (10 <sup>5</sup> cfu/10g)	Yeast (10 <sup>4</sup> cfu/10g)	Fungi (10 <sup>3</sup> cfu/10g)	Bacteria (10 <sup>5</sup> cfu/10g)	Yeast (10 <sup>4</sup> cfu/10g)	Fungi (10 <sup>3</sup> cfu/10g)
$T_1$	1.5	0	0	2.3	0.0	2.0	3.3	1.0	13.7	*	*	*
$T_2$				0.0	0.0	0.0	2.0	0.0	2.0	*	*	*
$T_3$				2.7	0.0	1.0	4.3	0.0	1.0	*	*	*
$T_4$				2.3	0.0	1.0	*	*	*	*	*	*
$T_5$				4.0	0.0	0.0	*	*	*	*	*	*
$T_6$				2.0	0.0	1.0	7.7	0.0	3.0	*	*	*
$T_7$				0.0	0.0	0.0	0.0	1.0	0	0	0	24.0
$T_8$				0.0	0.0	1.0	0	0.0	11.7	0	7.0	43.7
$T_9$				1.7	0.0	1.3	2.0	0.0	0	13.3	0.0	0.0

\*-unmarketable.



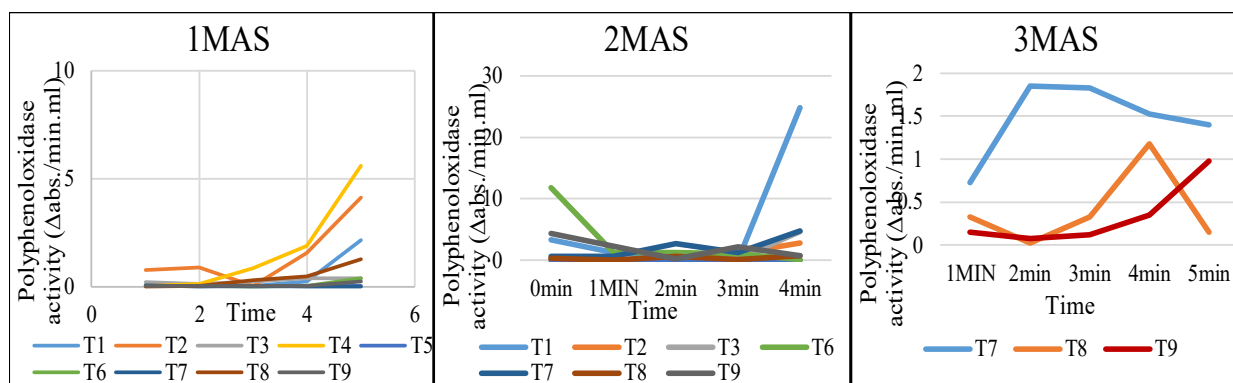


Fig 4: Polyphenoloxidase activity of frozen avocado slices during storage.

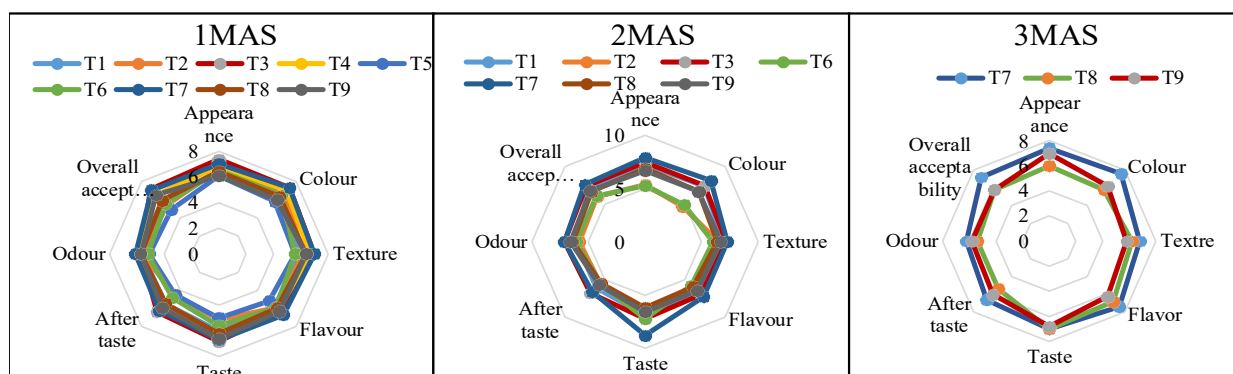


Fig 5: Total scores of organoleptic evaluation of frozen avocado slices during storage.



Fig 6: Frozen avocado slices at initial and treatments ( $T_7$ ,  $T_8$  and  $T_9$ ) after 3 months of storage.

fungus count was higher followed by bacteria which remained in the acceptable range (Table 1). Initially microbial count observed in fresh avocado sample was  $1.5 \times 10^5$  cfu/10 g bacteria. Bacterial population was not detected in  $T_7$  and  $T_8$  throughout the storage. In treatments  $T_2$ ,  $T_5$  and  $T_7$  fungal population were not detected after one month of storage. After 2 months no fungal count was seen in  $T_7$  and  $T_9$ . Yeast population ( $1 \times 10^4$  cfu/10 g) was observed only after two months of storage in  $T_1$  and  $T_7$ . After three months of storage yeast population ( $7 \times 10^4$  cfu/10 g) was observed in  $T_8$ . Frozen fruits are generally considered safe, with reduced water activity and microbial growth and thereby reduced spoilage caused by microbial activity (De Ancos *et al.* 2000).

### Organoleptic evaluation

After one month of storage of frozen avocado slices, highest consumer acceptance (54) was noticed in fruit slices treated

with  $T_3$  and  $T_7$  (Fig 6). Fruit slices treated with  $T_7$  retained significantly higher organoleptic scores throughout the storage (Fig 5). The acceptability of the frozen avocado slices decreased during storage due to the rancid taste, off-flavour and reduction in texture. 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.

### CONCLUSION

The study revealed that fresh avocado fruit slices, when pre-treated with 40% sucrose, 0.5% ascorbic acid and 0.1% potassium metabisulphite, before quick freezing to  $-20^\circ\text{C}$  within 30 minutes, can be preserved up to three months under frozen storage at  $-18^\circ\text{C}$  without much deterioration in physico-chemical, sensorial and microbiological quality.

Preservation of avocado fruit slices by freezing with appropriate food additives is a viable method of preservation of this delicate fruit with complex biochemical characteristics, in which conventional thermal processing can never be thought of as a viable method of preservation.

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