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Original Research Paper

Temperature induced biochemical changes and antioxidant activity in mature avocado (*persea americana* Mill.) fruit during storage

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ABSTRACT

The present study was carried out to determine the effect of different storage temperatures (5ºC, 9ºC, 12ºC and room temperature (26-32ºC) on biochemical and antioxidant properties of two avocado accessions (CHES-HA-I/I and CHES-HA-VII/I). The result showed that titratable acidity, total soluble solids, and protein content decreased, while, fat content increased with the advancement of storage. The higher antioxidant activity was recorded in fruits stored at 9ºC in both the accessions. At 5ºC, fruits exhibited signs of chilling injury and lower antioxidant activity. Significantly higher phenolic content was found in fruits stored at room temperature. It was observed that both antioxidants and total phenolic content of avocado fruits increased irrespective of storage temperatures. It is, therefore, concluded that unlike other tropical fruits, as the ripening progressed, avocado fruits exhibited major change in biochemical and antioxidant activity.

Keywords: Antioxidant activity, avocado, biochemical, low temperature, room temperature

INTRODUCTION

Avocado (*Persea americana* Mill.) is an important tropical fruit crop which is largely grown in Central American countries and now spreading to several tropical regions as well. Avocado is a rich source of fat and antioxidants. In addition to this, avocado fruits are also rich source of protein, carbohydrates (D-mannoheptulose), pigments, tannins, and phytoestrogens (Landahl et al., 2009). Temperature is one of the most important causes affecting biochemical and antioxidant activity in storage. It is reported that the relative rate of antioxidant activity decreased with variation in temperature (Reblova, 2012). The study of the ripening process at different storage temperatures is of great importance to generate information about the changes in biochemical, antioxidant activity and TPC during the storage. Therefore, the present study was undertaken to determine the change in biochemical composition, antioxidant activity, and TPC in the avocado fruit during storage.

MATERIALS AND METHODS

Based on yield potential, two avocado accessions *viz.* CHES-HA-I/I and CHES-HA-VII/I were selected from ICAR-Central Horticultural Experiment Station, Hirehalli, Tumakuru, Karnataka. The fruits were elongated (CHES-HA-I/I) to oval (CHES-HA-VII/I) shape and fruit weight varied from 300 to 500 grams. Fruits were harvested at optimum mature stage and brought to the laboratory. Later, these fruits were stored at low temperature (LT) 5 ºC, 9ºC, 12ºC and room temperature (RT) 26-32ºC.

Parameters such as titratable acidity (TA) was estimated by standard titrimetry method (AOAC, 2000), while, TSS (ºBrix) was measured by digital refractometer (ATAGO PAL-3, Japan). Protein content was estimated as per the procedure suggested by Lowry et al. (1951), whereas, fat content of avocado fruit was determined as per crude fat extraction method (Zulharmita *et al*., 2013) by using petroleum ether as a solvent. Antioxidant activities such as free radical scavenging activity were estimated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (Brand-Williams *et al*., 1995). FRAP (Ferric reducing ability of plasma) assay was done as per the method described by Benzie & Strain (1996). The concentration of total phenolic compounds was determined by the method described by Singleton et al. (1999). The statistical method as suggested by Panse and Sukhatme (1985) was employed to study the analysis of variance at 5% significance level.

RESULTS AND DISCUSSION

A gradual increase in TSS content of both the accessions was observed in RT storage. On 7th day, TSS increased from 9.00 to 10.90 ºB and from 9.67 to 11.26 ºB in CHES-HA-I/I and CHES-HA-VII/I, respectively (Table 1), whereas, in LT storage, TSS content increased slowly compared to RT storage. Among both the accessions, significantly higher TSS was recorded in accession CHES-HA-VII/I (Table 2). LT stored fruits were able to maintain higher TSS for longer time, this might be due to reduced respiration and slower biochemical reactions. These findings are in agreement with the results of Wills et al. (1989).

Table 1 : Effect of room temperature (26-32ºC) on TSS (ºBrix) and titratable acidity (%) of avocado fruit during storage

Room temperature	TSS (ºB)			Titratable Acidity (%)		
	Accession			Accession		
Storage period	CHES-HA-I/I	CHES-HA-VII/I	Mean	CHES-HA-I/I	CHES-HA-VII/I	Mean
Day 0	9.00	9.67	9.34	0.58	0.44	0.51
Day 2	9.97	10.67	10.32	0.43	0.27	0.35
Day 4	10.20	11.23	10.71	0.38	0.16	0.27
Day 7	10.90	11.26	11.08	0.14	0.12	0.13
Mean	10.02	10.71	-	0.38	0.24	-
	S.E.m±	C.D. (0.05)	-	S.E.m±	C.D. (0.05)	-
Accession	0.02	0.07	-	0.003	0.01	-
Storage days	0.05	0.14	-	0.01	0.02	-
Accession x Storage days	0.09	0.28	-	0.01	0.04	-

Table 2 : Effect of different low temperatures on TSS (ºBrix) and titratable acidity (%) of avocado fruit during storage

Storage Temperature	Storage Period	TSS (ºB)			Titratable Acidity (%)		
		Accession			Accession		
		CHES-HA-I/I	CHES-HA-VII/I	Mean	CHES-HA-I/I	CHES-HA-VII/I	Mean
5℃	Day 0	9.00	9.67	9.34	0.58	0.44	0.51
	Day 7	9.00	9.77	9.38	0.51	0.43	0.47
	Day 14	9.04	9.83	9.44	0.50	0.41	0.46
	Day 21	9.07	10.73	9.90	0.47	0.36	0.41
9℃	Day 0	9.00	9.67	9.34	0.58	0.44	0.51
	Day 7	9.3	9.93	9.62	0.49	0.42	0.46
	Day 14	9.43	11.67	10.55	0.40	0.16	0.28
	Day 21	9.60	11.97	10.78	0.34	0.14	0.24
12℃	Day 0	9.00	9.67	9.34	0.58	0.44	0.51
	Day 7	9.53	10.63	10.08	0.47	0.33	0.40
	Day 14	10.00	11.01	10.51	0.38	0.30	0.34
	Day 21	10.31	11.13	10.72	0.30	0.18	0.24
Mean		9.36	10.48	-	0.47	0.34	-
		S.E.m±	C.D. (0.05)	-	S.E.m±	C.D. (0.05)	-
Accession		0.01	0.03	-	0.001	0.004	-
Temperature		0.02	0.04	-	0.002	0.01	-
Storage days		0.02	0.06	-	0.003	0.01	-
Accession x Temperature		0.03	0.09	-	0.004	NS	-
Accession x Storage days		0.04	0.12	-	0.01	NS	-
Temperature x Storage days		0.06	0.18	-	0.01	0.02	-
Accession x Temperature x Storage days		0.12	0.35	-	0.02	NS	-

Table 3 : Effect of room temperature (26-32ºC) on protein and fat content of avocado fruit during storage

Room Temperature	Protein (%)			Fat (%)		
	Accession			Accession		
Storage period	CHES-HA-I/I	CHES-HA-VII/I	Mean	CHES-HA-I/I	CHES-HA-VII/I	Mean
Day 0	1.56	1.66	1.61	18.40	18.68	18.54
Day 2	1.35	1.44	1.40	18.62	18.99	18.80
Day 4	1.11	0.89	1.00	19.02	20.30	19.66
Day 7	0.75	0.50	0.63	20.44	20.14	20.29
Mean	1.19	1.12	-	19.12	19.53	-
	S.E.m±	C.D. (0.05)	-	S.E.m±	C.D. (0.05)	-
Accession	0.01	0.02	-	0.04	0.11	-
Storage days	0.01	0.04	-	0.08	0.23	-
Accession x storage days	0.03	0.08	-	0.15	0.46	-

Titratable acidity (TA) is an essential component for good flavour of the fruits and vegetables. A gradual decrease in TA was observed in all the storage temperature. Initially decrease in TA was at slower rate, from 4th day onwards, TA was declined rapidly up to 7 days of storage in RT. Among both the accessions, CHES-HA-I/I had slightly more acidity at the end of storage in RT. (Table 1). In LT storage, decease in TA was at slower rate. Among different low temperature storage, TA was not reduced much in fruits stored at 5ºC (Table 2). Decrease in TA under both the storage conditions might be due to utilization of organic compounds in the fruit respiration and other physiological processes. Wills et al. (1989) reported that acidity of fruits stored at low and ambient storage might be associated with the utilization of organic acids in respiration as respiratory substrate.

Table 4 : Effect of different low temperatures on protein and fat content of avocado fruit during storage

Storage Temperature	Storage Period	Protein (%)			Fat (%)		
		Accession			Accession		
		CHES-HA-I/I	CHES-HA-VII/I	Mean	CHES-HA-I/I	CHES-HA-VII/I	Mean
5ºC	Day 0	1.56	1.66	1.61	18.40	18.68	18.54
	Day 7	1.40	1.64	1.52	18.46	18.71	18.59
	Day 14	1.34	1.60	1.47	18.50	18.84	18.67
	Day 21	1.26	1.57	1.41	18.63	18.91	18.77
9ºC	Day 0	1.56	1.66	1.61	18.40	18.68	18.54
	Day 7	1.33	1.59	1.46	18.53	18.78	18.66
	Day 14	1.24	1.03	1.13	18.67	19.44	19.06
	Day 21	1.16	0.86	1.01	20.17	19.67	19.92
12ºC	Day 0	1.56	1.66	1.61	18.40	18.68	18.54
	Day 7	1.27	1.38	1.33	18.50	18.82	18.66
	Day 14	1.14	1.11	1.12	18.80	19.34	19.07
	Day 21	0.99	0.61	0.80	19.35	19.50	19.43
Mean		1.32	1.36	-	18.73	19 .00	-
		S.E.m±	C.D. (0.05)	-	S.E.m±	C.D. (0.05)	-
Accession		0.002	0.01	-	0.01	0.02	-
Temperature		0.003	0.01	-	0.01	0.03	-
Storage days		0.004	0.01	-	0.02	0.05	-
Accession x Temperature		0.01	0.02	-	0.02	NS	-
Accession x Storage days		0.01	0.02	-	0.03	0.09	-
Temperature x Storage days		0.01	0.03	-	0.05	0.14	-
Accession x Temperature x Storage days		0.02	0.07	-	0.10	NS	-

Protein content decreased as the storage days advanced. On the day of storage, protein content was 1.56% and 1.66% it reduced to 0.75% and 0.50% in CHES-HA-I/I and CHES-HA-VII/I respectively, on 7th day of storage at RT (Table 3). Under LT, significantly higher protein content was recoded at 5^{o}C. Among the accessions, CHES-HA-I/I had more protein content and decrease in protein was slower (Table 4). Decline in protein content during storage might be due to its use in fruit respiration and other metabolic processes. These results are in agreement with the findings of Singh & Dwivedi (2008).

Generally avocado fruits are rich source of fat and provide essential fatty acids needed for human body. At RT, higher fat content was recorded in accession CHES-HA-I/I (Table 3). Under LT storage, fat content in fruits stored at 5ºC was not increased significantly even after 21 days of storage, it might be due to chilling injury. Significantly more fat was found in fruits stored at 9ºC in both the accessions (Table 4). The increase in total fat during storage might be due to the synthesis of oleic acid. Eaks (1990) reported that rise in total lipids or fat content is primarily due to the synthesis of oleic acid. Richard et al. (2008) reported that there was a significant increase in total monounsaturated and saturated fatty acids and a decrease of polyunsaturated fatty acids during fruit ripening.

Significantly higher total phenolic content (TPC) was recorded in fruits stored at RT on 7th day (Table 5). This might be due to higher activity of enzymes which are responsible for production of phenolic compounds. Increase in TPC was found more in accession CHES-HA-I/I throughout the storage under RT. Among LT, 12ºC showed more TPC than 9ºC and maintained higher levels of these compounds on 21th day storage (Table 6). Increase in TPC attributed to various chemical and enzymatic changes during metabolic processes of fruits (Vieites *et al*., 2012). Storage at higher temperatures is not recommended for avocado because they can destroy the sensorial properties of the pulp by producing more phenolic compounds, mainly impairing colour and flavour.

The increase in DPPH and FRAP activity was more at RT on 7th day as compared to other storage temperatures (Table 5). Fruits stored at 5ºC showed lower rate of increase in DPPH activity. Fruits stored at 9ºC maintained significantly higher DPPH activity than 12ºC throughout the storage period (Table 6). This might be due to more synthesis of compounds like ascorbic acid, phenolic acids and flavonoid content. Antioxidant activities are strongly correlated with these compounds in fruits (Njus *et al*., 2020). At RT, on 7th day of storage, lowest FRAP activity was recorded in CHES-HA-VII/I compared to CHES-HA-I/I under RT (Table 5). At LT, highest FRAP activity was recorded in fruits stored at 9ºC on 21st day (Table 6). This might be due to presence of compounds like carotenoids, chlorophyll, etc. in the fruit which are responsible for higher antioxidant activity. These finding are in agreement with Reddy et al. (2011).

Table 5 : Effect of room temperature (26-32ºC) on total phenolic content and antioxidant activity of avocado fruit during storage

Room temperature	Total phenolic content (mg GAE/100g)			Antioxidant activity					
				DPPH (mg AAE/100g)			FRAP (mg AAE/100g)		
	Accession			Accession			Accession		
Storage period	CHES-HA-I/I	CHES-HA-VII/I	Mean	CHES-HA-I/I	CHES-HA-VII/I	Mean	CHES-HA-I/I	CHES-HA-VII/I	Mean
Day 0	24.49	11.30	17.89	15.50	20.00	17.75	21.69	20.77	21.23
Day 2	28.42	19.86	24.14	20.33	24.50	22.42	23.35	22.26	22.80
Day 4	36.35	35.44	35.89	26.00	50.83	38.42	24.58	28.06	26.32
Day 7	76.07	45.70	60.88	53.17	46.09	49.63	32.02	25.16	28.59
Mean	41.33	28.07	-	28.75	35.36	-	25.41	24.06	-
	S.E.m±	C.D. (0.05)	-	S.E.m±	C.D. (0.05)	-	S.E.m±	C.D. (0.05)	-
Accession	0.18	0.55	-	0.15	0.45	-	0.07	0.22	-
Storage days	0.37	1.10	-	0.30	0.90	-	0.15	0.44	-
Accession x Storage days	0.73	2.20	-	0.60	1.81	-	0.30	0.88	-

Table 6 : Effect of different low temperatures on total phenolic content and antioxidant activity of avocado fruit during storage

Storage temperature	Storage period	Total phenolic content (mg GAE/100g)			Antioxidant activity: DPPH (mg AAE/100g)			Antioxidant activity: FRAP (mg AAE/100g)		
		Accession			Accession			Accession		
		CHES-HA-I/I	CHES-HA-VII/I	Mean	CHES-HA-I/I	CHES-HA-VII/I	Mean	CHES-HA-I/I	CHES-HA-VII/I	Mean
5ºC	Day 0	24.49	11.30	17.89	15.50	20.00	17.75	21.69	20.77	21.23
	Day 7	27.33	17.96	22.65	17.83	22.11	19.97	22.07	20.85	21.46
	Day 14	36.67	18.70	27.68	18.33	25.58	21.96	22.38	23.08	22.73
	Day 21	40.00	20.32	30.16	20.95	28.11	24.53	23.26	25.55	24.41
9ºC	Day 0	24.49	11.30	17.89	15.50	20.00	17.75	21.69	20.77	21.23
	Day 7	28.63	18.17	23.40	18.50	24.68	21.59	23.81	21.12	22.46
	Day 14	29.47	33.68	31.58	63.43	42.89	53.16	37.14	32.54	34.84
	Day 21	32.56	39.30	35.93	69.34	47.59	58.46	49.50	41.58	45.54
12ºC	Day 0	24.49	11.30	17.89	15.50	20.00	17.75	21.69	20.77	21.23
	Day 7	31.61	19.68	25.65	19.19	41.76	30.48	24.21	29.41	26.81
	Day 14	34.10	35.47	34.79	56.95	42.90	49.93	33.14	32.93	33.03
	Day 21	35.17	45.89	40.53	64.89	45.16	55.03	43.33	38.69	41.01
Mean		30.75	23.59	-	32.99	31.73	-	28.66	27.34	-
		S.E.m±	C.D. (0.05)	-	S.E.m±	C.D. (0.05)	-	S.E.m±	C.D. (0.05)	-
Accession		0.08	0.22	-	0.06	0.17	-	0.03	0.09	-
Temperature		0.12	0.34	-	0.09	0.26	-	0.05	0.13	-
Storage days		0.16	0.45	-	0.12	0.35	-	0.06	0.17	-
Accession x Temperature		0.24	0.67	-	0.18	0.52	-	0.09	0.26	-
Accession x Storage days		0.32	0.90	-	0.24	0.70	-	0.12	0.35	-
Temperature x Storage days		0.47	1.35	-	0.37	1.04	-	0.18	0.52	-
Accession x Temperature x Storage days		0.95	2.70	-	0.73	2.09	-	0.37	1.05	-

CONCLUSION

From the present study, it was concluded that, room temperature had significant effect on surging biochemical processes. Among different low temperatures, 9ºC was found to be ideal for storage of fruits followed by 12ºC. Avocado fruits stored at 5ºC failed to ripe due to chilling injury. Significantly higher TPC was found in fruits stored at RT. It is suggested that storing of avocado fruits at 9ºC would help to extend the shelf life with better fruit quality. Among the accessions, CHES-HA-I/I can be stored for longer period (7 days and 21 days at RT and 9ºC, respectively) with higher fruit quality and least spoilage as compared to CHES-HA-VII/I.

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