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EFFECT OF WAXING ON FREE AMINO ACIDS OF KINNOW MANDARINS (*CITRUS RETICULATA* BALNCO) DURING STORAGE

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Free amino acids identified in kinnow juice in decreasing order of concentration were serine, aspartic acid, glutamic acid, arginine, threonine, proline, lysine, alanine, valine, cystine, glycine, phenylalanine, tyrosine, histidine, leucine, isoleucine and methionine. Preliminary investigations revealed that there was no effect of waxing on free amino acids of kinnows. However, there was considerable decrease in most of the amino acids during postharvest storage at room conditions (12-26°, 68-90% RH). Further studies are needed in this direction to understand the metabolism of free amino acids and their relation to internal quality deterioration during postharvest handling of citrus fruit.

Key words: Waxing, Newspaper lining, Free amino acids.

INTRODUCTION

Citrus fruits can metabolize glucose [1] and malic acid [2] through pyruvic acid to acetaldehyde and ethanol. During controlled atmosphere (CA) storage the increase in ethanol correlated to the decrease in Brix and acid in grapefruits but in Pineapple and Valencia oranges these changes did not correlate [3]. This indicates that Brix and acid are not necessarily the only source of ethanol (responsible for abnormal flavour development) in all the citrus cultivars. Amino acids have been reported to be the precusors of ethanol and volatile compounds in ripening fruits [4], and glutamate from protein breakdown was suggested as a respiratory substrate in lemons [5]. Therefore, amino acids and protein may be metabolized and contribute to the ethanol production during postharvest storage of citrus fruits.

Free amino acids are the largest fraction (70 %) within the nitrogenous compounds in citrus fruits [6, 7, 8]. Total free amino acids change during maturation [9, 10] and individual amino acids have been indicated to go through maxima and minima independently during ripening process [11]. Brix/acid ratio x arginine as maturity index has been reported to be more closely correlated with sensory quality than just the brix/acid ratio [12]. Reduction in free amino acid contents of infected [13] and stored [3] citrus fruits have also been reported. The present preliminary study, therefore, was undertaken to investigate changes in free amino acid contents of waxed kinnow mandarins (*Citrus reticulata* Blanco) stored at room conditions.

MATERIALS AND METHODS

Kinnow mandarins were procured from garden near Faisalabad in March and transported by road to the laboratory in wooden boxes lined with newspaper sheets. The stems were cut close to the shoulders and bruised and injured fruits were discarded. Fruits were washed in running tap water and air-dried on the wire trays. All the kinnows were dipped for two min. in aqueous suspension of 1000 ppm thiabendazole (TBZ). The fruits were divided into two equal lots. One lot of kinnows was coated with full strength commercial wax emulsion (Britex-561, procured from Florida, USA) while the second lot was not further treated. Newspaper sheets (0.093 mm) were used as lining material for waxed and TBZ only treated fruits. Waxed and unwaxed fruits were separately packed in perforated wooden boxes (36 x 30 x 18 cm size with 3 slits of 1 cm) and stored at ordinary room conditions (12-26°, 68-90 % RH).

Individual free amino acids of juice of waxed and unwaxed kinnows were measured with LKB 4101 amino acid analyser [14]. Juice was extracted with manually operated pressure type citrus juice extractor and passed through double folded muslin cloth. The samples were clarified before analysis by centrifugation at 50,000 x g for 20 min. followed by filtration through Whatman No. 44 filter paper. 0.5 ml diluted juice (dilution factor 10) was injected into the instrument and various amino acids were eluted with a sodium, three buffer system. The amino acids were identified by comparing the resulting peaks with those of standard acids. The quantity of each amino acid was

calculated from the area under their respective curve by the following formula:

$$\text{Concentration of unknown} = \frac{\text{Area of unknown}}{\text{Area of standard}} \times \text{concentration of standard.}$$

The results (average of two runs) were expressed as mg amino acids per 100 ml juice and μmoles per ml juice.

RESULTS AND DISCUSSION

Results regarding the free amino acid contents of fresh kinnows and those of control and waxed fruits after 3 weeks storage at room conditions are presented in Table. Free amino acids identified in fresh kinnow juice in decreasing order of concentration were: serine, aspartic acid, glutamic acid, arginine, threonine, proline, lysine, alanine, valine, cystine, glycine, phenylalanine, tyrosine, histidine, leucine, isoleucine and methionine. Data on free amino acid contents of citrus cultivars grown in Pakistan is very scant. Some information, however, regarding free amino acids of Pakistani citrus fruits has been reported by Elahi and Khan [15]. Nine amino acids identified by them from fresh kinnow juice in decreassing order of concentration were: valine, tyrosine, proline, serine, arginine, aspartic acid, threonine, lysine and alanine. Most of the concentrations of amino acids reported by them are not in agreement with present findings. Most probable reason for this discrepancy could be the difference in analytical techniques used. In present studies amino acids were determined in an amino acid analyser by ion-exchange chromatography [16] whereas paper chromatography was used by Elahi and Khan [15]. Choice of buffer system for elution of amino acids of physiological fluids is debatable. Sodium system was used in the present studies as this system is less sensitive to variation than the lithium system. Moreover, lithium system is only justified if simultaneous separation of two amines-glutamine, asparagine and their corresponding acids-glutamic acid and aspartic acid is required [17].

There is a lot of variation in results on free amino acid contents of citrus fruits reported by various workers from different parts of the world. This variation, in addition to type of analytical technique used, might be due to varietal differences or to cultural or environmental factor [12, 15, 18, 19, 20, 21] type of extraction, finishing and washing (processing), and storage of fruit juice before analysis may also be responsible for these differences [19, 22, 23].

It can be noted from results in Table 1 that free amino acid contents of control and waxed kinnows after 3 weeks storage were almost comparable. However, there was decrease in most of the amino acids during storage at room conditions. Great differences were seen in serine, arginine, proline valine, cystine and methionine, content of fresh and stored kinnows. The differences were relatively less in case of aspartic acid, threonine, tyrosine, leucine and isoleucine whereas values of glutamic acid, lysine, alanine, glycine, phenylalanine and histidine were almost comparable in fresh and in 3 week old kinnows.

Being nonclimateric in nature, citrus fruits ripen on the tree, hence their maturity standards are synonymous with internal quality standards [24, 25, 26]. Results, however, on changes in different amino acids during maturation are inconsistent and contradictory [6, 9, 10, 27, 28, 29]. Results on changes in free amino acid contents during postharvest storage of citrus fruits are very scarce. Infection of Mosambi oranges with *Botrydiplodia theobromea* resulted in reduction of free amino acid contents. Arginine contents were reduced to about one third whereas proline had totally disappeared [13]. Vandercook *et al.* [22] showed that the total amino acids decreased significantly in lemons stored for 15 weeks. In a subsequent lemon storage study, however, it was shown that the mol. percentage of the individual amino acids did not change over a period of 180 days [20].

Results reported on changes in free amino acids during ripening and senescence of other fruits are also inconsistent. Gortner and Singleton [30] noted that in pineapple fruit glycine and alanine concentrations were both moderate during prematuration, low during maturation, moderate again during ripening and large during senescence. Valine was present in higher concentration only during early development and during senescence. Level of methionine increase only at the time of ripening. Arginine and proline were only found on occassions at low levels. Vines and Grierson [31] reported a consistent decrease in amino acids during ripening of carambola fruits, coupled with an increase in a-ketoglutaric acid suggesting utilization of amino acids as respiratory substrate. Krishnamurthy *et al.* [32] observed that aspartic acid and glutamic acid concentration were high in early stages of ripening in Pairi and Alphanso mangoes but suddenly diminished at the respiration climateric. In another study Krishnamurthy and Subramanyam [33] found decrease in aspartic acid, threonine, serine, glutamic acid, phenylalanine, lysine and arginine during ripening of Alphonso mangoes, but proline, glycine and alanine increased. Shashirekha and Patwardhan [34] found in Alphonso mangoes 6 amino acids: aspartic, glutamic, alanine, glycine, arginine and lysine: all of them except alanine decreased during ripening.

Table 1.Effect of waxing on free amino acid contents of kinnow mandarins stored at room conditions (12-26°C, 68-90 % RH)

Amino acids (juice)	Fresh fruit		3 week			
			Control		Waxed	
	mg/100 ml	μmoles/ml	mg/100 ml	μmoles/ml	mg/100 ml	μmoles/ml
Aspartic acid	21.21	1.590	18.11	1.360	17.95	1.350
Threonine	18.62	1.560	13.43	1.130	14.73	1.240
Serine	29.50	2.810	8.84	0.840	6.05	0.570
Glutamic acid	19.18	1.300	20.12	1.370	18.15	1.240
Proline	18.44	1.600	5.39	0.470	4.57	0.390
Glycine	1.18	0.260	0.93	0.124	1.16	0.154
Alanine	7.46	0.840	8.23	0.920	6.87	0.770
Cystine	1.19	0.098	0.05	0.004	0.08	0.007
Valine	3.86	0.290	2.64	0.198	2.62	0.197
Methionine	0.36	0.024	0.21	0.014	0.12	0.008
Isoleucine	0.70	0.053	0.55	0.042	0.65	0.049
Leucine	0.85	0.065	0.72	0.055	0.71	0.054
Tyrosine	0.95	0.052	0.87	0.048	0.63	0.035
Phenylalanine	1.08	0.065	0.99	0.060	1.04	0.063
Lysine	8.70	0.590	8.30	0.570	7.45	0.510
Histidine	0.90	0.058	0.95	0.062	0.85	0.055
Arginine	19.08	1.096	6.14	0.350	5.55	0.320

All the values are average of two runs.

Present results of preliminary postharvest storage experiment on kinnows and reports of other workers on citrus and other fruits do not elucidate consistent changes in free amino acid contents. More detailed studies including various cultivars with different post-harvest treatments and storage environments are suggested to establish any possible relationship between changes in free amino acids and deterioration of internal quality of citrus fruits.

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