



The formation of a tertiary carbonium ion is the driving force of this hydrolysis. Replacement of the carbonyl by a thiocarbonyl group appears to enhance the rate of dissociation, since 4 was found to be very unstable.

CONCLUSIONS

O-*tert*-Butyl monothiocarbonate thiol esters are slowly hydrolyzed in hot aqueous medium to give the corresponding thiols. Probably partly due to their lower volatility, the precursors have threshold values which are generally more than ten times higher than those of the thiols. They can therefore be added to foodstuffs below their threshold values and still generate the thiols in organoleptically discernible amounts. Examples of some applications are described in a pending patent (van der Heijden and Schutte, 1971).

The *O*-*tert*-butyl moiety may be replaced by any other tertiary alcohol group. This makes the purification after separation more difficult, since the volatility of the thiocarbonate decreases with increasing size of the alcohol

grouping. On the other hand, this diminished volatility may be advantageous for the release of the thiol, as the thiocarbonate is evaporated from the food with even more difficulty and therefore has more chance to hydrolyze. Indeed, the formation of thiols from *O*-*tert*-hexyl esters 7 and 8 proceeded even more readily than from the corresponding *O*-*tert*-butyl esters 5 and 9.

The LD₅₀ values of compounds 5 and 16 for mice were found to be 3.67 and 0.58 ml/kg, respectively. These values do not preclude usage of these compounds in food.

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Physicochemical Changes in Some Pakistani Mango Varieties during Storage Ripening

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Some physicochemical changes in four popular Pakistani mango varieties during storage ripening have been studied. Acidity decreases during ripening with the simultaneous increase in reducing sugars. Formol value, nonreducing sugars, after reaching a peak after 4 days, start decreasing. The same 12 free amino acids have been identified and estimated in all the varieties. Both total

and individual amino acids increase in concentration in the early ripening period and then decrease. Malda variety is the richest in proteins, amino acids, and sugars and possesses the least acidity. The organoleptic maturity coincides closely with the peak values of amino acids and sugars after 4-8 days during ripening.

Mango is known as the king of table fruits and is universally popular due to its pleasant flavor and healthful dietetic qualities. From the point of view of acreage and production, it is one of the most outstanding of all Pakistani fruits. Many varieties are available in the market, and endeavors are being made to develop newer varieties. These varieties differ in flavor, nutritional qualities, and rate of spoilage under similar storage conditions. A large

fraction of the total production is spoiled due to over-ripening and ultimate fermentative breakdown of carbohydrates.

A study of the changes occurring during ripening will yield useful information which would serve a dual purpose. It would help the agriculturists to develop new varieties with better overall qualities by cross-breeding and it would help the food technologists to estimate maturity and develop better preservation techniques.

Studies on changes in chemical composition during ripening of some varieties of mangoes of the Indo-Pakistan subcontinent have been reported (Basu *et al.*, 1947; Cheema *et al.*, 1950; Lely *et al.*, 1940; Srivastaga, 1953; Wahab and Khan, 1954). This work was undertaken to study the

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chemical changes during ripening of newly developed popular Pakistani mango varieties.

EXPERIMENTAL SECTION

Reagents and Apparatus. Solvents used were of analytical grade. Ninhydrin reagent was prepared by dissolving 0.5 g of commercial ninhydrin in 100 ml of acetone. pH was measured by a pH meter (Model 23A, Electronic Instruments, Ltd., Richmond Surrey). Paper chromatography was carried out on Whatman No. 1 paper using a glass chamber (21 × 8 in.). Optical density readings were taken with a Beckman DU spectrophotometer.

PROCEDURE

Preparation of Samples. Four mango varieties, *viz.* Malda, Anwar Ratual, Katha (Tukhmi), and Dusehri, were arranged from Buchha Garden, located about 12 miles from Lahore. Mangoes were harvested at the unripened stage from trees of respective varieties on July 13, 1970. Each variety was stored in hard paper boxes separately in the laboratories at room temperature, fluctuating between 30 and 34°. All the physical and chemical studies were made after an interval of 4 days, starting from the time of harvesting.

Physical changes such as color were observed visually and texture was noted by pressing with fingers. For chemical changes, the skin of the mangoes was removed and 50 g of the edible portion of each variety was well macerated in a pestle and mortar and was quantitatively transferred to a 100-ml measuring flask by washing with distilled water. The final volume was made to 100 ml. It was well shaken and allowed to stand for sedimentation of fibrous material. All the determinations except moisture and free amino acids were carried out on the supernatant liquid. Determination of moisture, dry matter, pH, acidity (as citric acid), and reducing and nonreducing sugars was carried out using the standard methods of the AOAC (1960). Formol value was estimated by Safina's method (Safina, 1964). The results are shown in Table I.

Extraction and Chromatographic Separation of Free Amino Acids. The procedure adopted for extraction of amino acids is essentially the same as reported in literature (Pant and Tulsiani, 1968). The well macerated edible portion (10 g) was extracted without defatting with 30 ml of 70% ethanol for 30 min and centrifuged, and the supernatant layer was decanted. The residue was repeatedly extracted with 70% ethanol until the supernatant layer gives no coloration with ninhydrin reagent. All the super-

natant layers were combined, concentrated under vacuum, and made to a definite volume with distilled water.

The solution thus obtained is used for both qualitative and quantitative determination of free amino acids using paper chromatography according to the method described earlier (Elahi and Khan, 1971).

Total free amino acids were estimated by mixing 0.5 ml of ninhydrin solution with 0.5 ml of each extract separately in test tubes and diluting to 10 ml with ethanol. The tubes were then kept at 70° for 30 min and the intensity of the color was read at 570 m μ , with a reagent blank and ethanol (0.5 and 9.5 ml, respectively) using Beckman DU spectrophotometer.

The concentration of both total and individual free amino acids was then estimated in terms of alanine from a standard curve prepared for it. The results of qualitative and quantitative determinations have been included in Table II.

DISCUSSION

Mangoes of all four varieties possess hard texture, green skin, light yellow edible portion, bland flavor, and sour taste at the time of picking. With time, these varieties undergo changes both externally and internally. After a storage period of 4 and 8 days, the skin color changes to greenish-yellow to yellow, the edible portion turns golden to orange (with the intensity of color increasing toward the stone), sourness diminishes, and a sweet taste and pleasing flavor develop. After this storage period all of the varieties except Anwar Ratual over-ripen, which is accompanied with loss in flavor and development of off-color of both skin and edible portion; thus organoleptically they are rendered unacceptable. Anwar Ratual possesses acceptable organoleptic qualities even after 12 days of storage time.

Table I shows that the dry matter decreases during ripening under storage, which may be due to an increase in moisture content of the edible portion. These results are in agreement with those reported in literature (Lely *et al.*, 1940). At the time of picking the Katha variety has maximum acidity and the Malda variety has the minimum. It will be observed that the decrease in acidity results in a simultaneous increase in sugars. The acidity decreases and reducing sugars increase continuously during ripening. Total and nonreducing sugars start declining after reaching a peak value after 4 days storage of all the varieties except Anwar Ratual, which attains maximum values after 8 days. At the time of picking, the reducing

Table I. Chemical Changes during Ripening of Some Mango Varieties

Mango variety	Storage period, days	Moisture, %	Dry matter, %	pH	Acidity, %	Reducing sugars, %	Nonreducing sugars, %	Total sugars as reducing sugars, %	Formol value, (ml of molar NaOH/1000 g)
Malda	0	77.6	22.4	3.62	0.84	8.26	2.16	5.42	18.4
	4	81.8	18.2	4.40	0.464	4.54	10.53	15.07	25.2
	8	82.08	17.92	5.60	0.112	5.26	9.15	14.41	17.6
	12	83.40	16.60	6.50	0.003	5.85	8.85	14.70	12.6
Anwar Ratual	0	74.6	25.40	3.10	2.12	2.3	1.85	4.15	12.14
	4	80.2	19.80	3.50	1.06	3.62	4.3	7.92	18.20
	8	80.89	19.16	4.30	0.39	4.62	10.01	14.63	11.85
	12	81.87	18.13	5.25	0.017	5.14	8.89	14.03	11.46
Katha (Tukhmi)	0	79.0	21.0	3.0	2.96	1.64	1.57	3.40	11.80
	4	82.2	17.8	4.05	0.75	2.60	10.26	12.86	19.20
	8	83.5	16.5	4.80	0.28	2.66	9.49	12.15	15.20
	12	83.9	16.1	4.95	0.023	4.34	7.25	11.61	11.15
Dusehri	0	78.6	21.40	3.40	1.08	3.44	2.4	5.84	8.30
	4	80.7	19.30	4.05	0.56	3.62	11.03	14.65	15.37
	8	81.76	18.24	5.20	0.112	4.85	10.34	15.19	11.28
	12	82.1	17.90	5.80	0.007	5.46	9.02	14.48	9.37

Table II. Quantitative Estimation of Free Amino Acids in Some Mango Varieties during Ripening

Mango variety	Storage ripening period, days	Total amino acids	Amino acids, mg % of edible portion											
			I ^a	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Malda	0	294	8	30	21	8	28	15	35		60	45	44	
	4	428	22	38	40	17	51	35	42	12	79	51	52	15
	8	277	17	20	14	12	25	31	27	12	45	28	31	10
	12	214	14	18	11	9	20	25	21	9	37	22	23	10
Anwar Ratual	0	189	5	27	16	8	19	10	31		45	26	22	
	4	304	14	32	19	13	35	18	32	7	58	32	37	13
	8	193	11	24	15	10	18	13	30	7	30	15	18	8
	12	186	11	20	15	9	18	13	26	7	30	15	15	8
Katha (Tukhmi)	0	205		23	16	6	17	8	27		38	21	25	
	4	318	18	29	33	9	31	17	37	8	51	36	40	14
	8	238	13	18	24	9	21	14	25	6	43	24	29	12
	12	184	10	14	15	8	20	11	19	6	39	18	19	8
Dusehri	0	144	8	24	15	6	20				30	18	9	
	4	241	13	29	19	8	30	14	14	8	46	29	30	8
	8	189	11	21	17	7	22	12	14	7	36	22	19	7
	12	175	8	21	17	7	19	12	13	7	37	19	17	7

^aI, phenylalanine. II, aspartic acid. III, glutamic acid. IV, tyrosine. V, cystine. VI, arginine. VII, valine. VIII, methionine. IX, alanine. X, serine. XI, lysine. XII, leucine. Asparagine, threonine, glycine, proline, and histidine were not found in any of the mango varieties.

sugars are greater than the nonreducing ones, and during ripening the latter increase at a much faster rate. The continuous increase in the reducing sugars may be apparently due to the breakdown of nonreducing ones. The formol value increases up to 4 days and then decreases in all the varieties.

Table II shows the qualitative and quantitative study of the free amino acids in each mango variety during storage ripening. The same 12 amino acids have been identified in all the varieties. It will be observed that these mango varieties differ in the total number of amino acids at the time of picking. Thus, Dusehri contains the minimum number (*i.e.*, eight) and both Malda and Anwar Ratual possess the maximum number (*i.e.*, ten each). It is interesting to note that methionine and leucine are not present in all the varieties, and in addition to these acids arginine and valine have not been identified in Dusehri and phenylalanine has not been identified in Katha at the time of picking. It will not be impertinent to mention that the following pairs of acids are not separable in any of the solvent mixtures used: leucine and isoleucine and cystine and cysteine. These have been identified and estimated in terms of leucine and cystine, respectively.

The quantitative study of both total and individual free amino acids in each variety during ripening indicates that the Malda variety is richer in both total and individual free amino acids than the others. Both total and individual acids increase during the early ripening period up to 4 days and then decrease as the fruits become ripe to over-ripe. It is interesting to note that although the same essential amino acids (*i.e.*, lysine, leucine, valine, arginine, and phenylalanine) are present in all the varieties, Malda contains them in higher concentrations than the others during the ripening period. Lysine is the most abundant essential amino acid in all the varieties.

It is clear from Tables I and II that nonreducing and total sugars, formol value, total and individual free amino acids increase up to 4 days and then decrease. The fruits

attain organoleptic maturity at the peak values of the above-mentioned factors. The rise and fall in these factors may be used as an index of maturity for these mango varieties.

The rate of rise and fall of sugars and free amino acids is not uniform in all the varieties, as is evident from Tables I and II. Table II shows that all the mango varieties are qualitatively similar but quantitatively dissimilar, as the same 12 amino acids are present in different quantities. Although the overall reaction in each variety may be of the same nature, the difference in the above-mentioned factors may be attributed to the difference in the rate and pathway of enzymic reactions from variety to variety. This possibility has been shown in some varieties of certain fruits (Qudrat-i-Khuda *et al.*, 1961; De and Debnath, 1966).

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