Malonic Acid, a Proposed Indicator of Orange Fruit Senescence

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Summary. Malonic acid was found to accumulate in peel tissues and in juice of oranges during maturation and storage on shelf. Its accumulation was correlated with textural parameters of senescence. Although the reasons for this behaviour are not clear, malonic acid can be regarded as a reliable indicator of orange fruit senescence.

While the onset of senescence of climacteric fruits is rather clearly defined by the occurrence of characteristic physiological events, it is difficult to determine when the so-called non-climacteric fruits enter the final phase of senescence and tissue degradation, though for citrus fruits changes in peel pigments may be considered as an initial step in these processes[2].

During a search for non-hormonal parameters suitable to define the first stages of senescence of Shamouti oranges (*Citrus sinensis* L., Osbeck), organic acids of different fruits parts were quantitatively determined by GLC of their methylated products[3].

Fruits from 2 field experiments (2 locations, different irrigation schedules and/or a treatment by synthetic auxin + gibberellic acid A_3 at an early stage of fruit life) were picked at the middle of the conventional harvest season (January, February) and stored under 'shelf' conditions (17°C, varying air humidity) for 3 months, a period about 2–3 times longer than usual for shipping and marketing. During this period several determinations of qualitative and quantitative acid composition were carried out along with other physical and chemical parameters.

Organic acids of juice and of peel (analyzed both in the external coloured *flavedo* and the internal whitish spongy mesocarp, *albedo*), beyond variability caused by previous treatments in the field, showed clear-cut developmental patterns with fruit ageing under shelf conditions.

The concentrations of some acids, notably citric in all tissues and malic in juice, decreased during this period. Some others behaved irregularly with regard to time and different tissues. Malonic acid could be singled out, as its concentration increased in all cases during the storage period, along with fruit ageing.

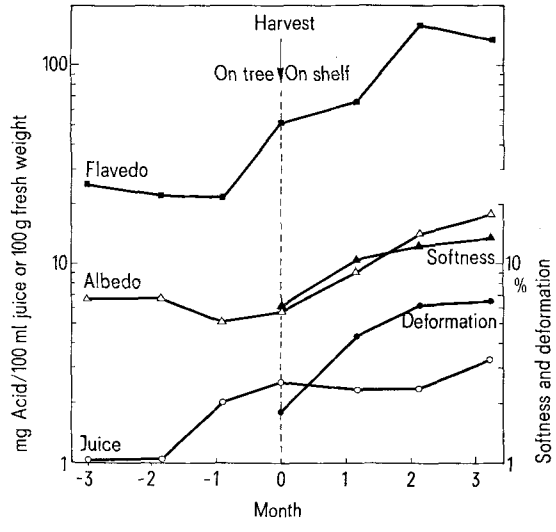
The Figure shows malonic acid concentration from early maturity, before picking, to full senescence under shelf conditions. A very marked increase in concentration occurs in flavedo layers after picking and during storage. Concentrations in this tissue are comparatively very large and fluctuate around 50–60% of total organic acids detected during the period after picking, but attain only about 45% to 46% while fruit is still on the tree.

Flavedo layers are composed of relatively small cells which retain until late in fruit life mitotic capacities[4] as expected of outermost layers which must continue to expand under the thrust of growing endocarp. They are important centers of specific synthetic activities and produce different metabolites, e.g. lipids of natural cuticle wax[5], ascorbic acid[6], etc. They also accumulate more malonic acid than other fruit tissues.

The thicker spongy tissues of albedo show a clear increase in malonic acid concentration beginning at harvest time. Malonic acid also progressively increases in percent of total organic acid concentration from values 29–31% before harvest up to almost 67% at the end of the storage period.

Albedo layers build up at least $^2/_3$ of total Shamouti peel thickness, i.e. they are about 4–5 mm thick. Due to their spongy texture and high pectin content[7], they are prone to deformation and collapse, as it happens in many varieties by creasing[8]. The Figure also shows softness and deformability of fruits as assessed by the Cornell pressure tester[9]. The former parameter is obtained by compressing fruit for 30 sec with a 3 kg weight, the latter by subtracting recovery after 30 additional sec, both in percent of fruit cross diameter. These parameters provide an evaluation of fruit deterioration due to water loss and weakening of tissues. The parallelism between malonic acid concentration in albedo and these textural parameters is evident. Our data for softness and malonic acid concentration are highly significantly correlated ($r = 0.88^{**}$).

Concentration in juice is much smaller: here, maximum values of malonic acid (which are found as above at the end of storage) are only about 0.2% of total organic acids



Malonic acid content of peel layers (flavedo, albedo) and of juice of oranges while still on tree and its accumulation after harvest under prolonged shelf storage, as well as softness and deformation of fruit under prolonged shelf storage.

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content. The relative increase during the ageing period is due mainly to a decrease in other acids (especially citric and malic).

Juice vesicles have been found to contain all the enzymes of TCA and of some auxiliary cycles from an early date[10]; juice at maturity contains very large amounts of citric and malic acids; in comparison malonic acid is only a negligible fraction, though it tends to accumulate with age.

The trend of malonic acid accumulation from incipient senescence onwards is therefore common to all these tissues, notwithstanding their widely different capabilities. Our data confirm previous findings of CLEMENTS[11] related to peel of Washington Navel oranges during maturation and delayed picking. Due to less sensitive methods, however, he was unable to detect malonic acid in juice. Malonic acid is a recognized competitive inhibitor of succinic dehydrogenase, blocking the classical TCA cycle. The concentrations we found in flavedo tissues are similar to those causing malonate inhibition of 0_2 uptake in vitro (1 to $5 \times 10^{-2} M$)[12]. Also pH values of peel tissues are pH 5–5.5, i.e. similar to those needed for the reaction in vitro[12]. On the other hand, malonic acid is probably located in vacuoles away from mitochondria. Malonic acid through its activated derivative malonyl-CoA is active in many metabolic paths (as lipid and phenolic biosyntheses, etc.) and its accumulation could also be connected with slowing down of biosynthetic activities at incipient senescence.

Although the reasons for malonic acid accumulation have not yet been explained, such accumulation seems to provide a reliable and adequate indicator of fruit tissue senescence in oranges.

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Changes in Blood Tryptophan Level During Sleep Deprivation

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Summary. Prolonged sleep deprivation elicits a significant elevation of the plasma level of free tryptophan which appears to be involved in increased excretion of 5-HIAA during this state through enhanced 5-HT synthesis.

In an earlier paper we have demonstrated that sleep deprivation (SD) is associated with changes in the excretion of the end-product of serotonin metabolism (5-HT), i.e. 5-hydroxyindole acetic acid (5-HIAA)[3].

Temporary elevation of 5-HIAA excretion on days 2 and 3 of SD was explained by an increased 5-HT release from tissues, on one hand, and by a stress manifestation of SD, on the other. Latest investigations showed that changes in blood tryptophan (TP) levels[4], particularly in the free tryptophan[5–7], may be a reliable index of the changes of 5-HT synthesis. We therefore investigated the changes of blood TP during SD.

Method. 6 healthy volunteers (20–23 years) were observed for total, free and bound TP during 120-hour SD and during 2 control periods under experimental conditions described in the preceding paper[3]. The plasma TP was measured with the spectrofluorometric method according to ECLESTONE[8], the free TP was filtered before processing through a 2 100 CF 50 membrane filter. Heparinized blood was withdrawn at 06.00 h and at 18.00 h and the average of the 2 values was used for the final evaluation.

Results and discussion. The Table shows that both the free and bound TP increase during SD. However, this increase is significant only for the free fraction, reaching its

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		1st control period	Sleep deprivation (h)				2nd control period	
			24–48	48–72	72–96	96–120	48–72	72–96
Bound tryptophan	µg/ml	10.58	12.117	11.8	11.77	9.94	11.158	11.158
	S.D.	2.31	2.871	2.189	2.77	1.51	1.8	1.88
	n	30	12	12	12	12	12	12
Free tryptophan	µg/ml	2.507	2.6	3.67[c]	3.6[c]	3.57[c]	3.317[a]	3.23[a]
	S.D.	0.78	0.83	0.93	0.93	0.49	1.23	0.75
	n	30	12	12	12	12	12	12

n, number of observation; [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$.