

CHANGES IN PROTEIN PROFILES IN ARIL OF *LITCHI CHINENSIS* SONN DURING FRUIT RIPENING AND SENESCENCE

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Colorimetric and electrophoretic studies on protein profiles in aril of *Litchi chinensis* Sonn. cv. Deshi were made at various stages of fruit ripening and senescence. The protein content tended to parallel the total concentration of electrophoretically separated protein bands except at advanced senescence. Five electrophoretically separable protein bands of widely different charge and size were detectable exhibiting fluctuations in their pattern and relative concentrations at different stages of fruit ripening and senescence.

Key Words : *Litchi chinensis* Sonn. aril PAGE protein ripening senescence.

Fruit ripening is dependent on synthesis of both RNA and protein (Brady, 1987). Ripening is accompanied with such changes as the decline in total protein, diminution of its synthesis and enzymatic degradation of proteins, which eventually lead to qualitative and quantitative changes in protein components. Information on protein patterns of fruits has been scanty (Clements, 1970; Parups, 1971). Although protein content had previously been reported by Sah *et al.* (1984) to rise in course of ripening of *Litchi chinensis* Sonn., the protein patterns and their correlation with total protein have not been investigated so far, which is the object of the present study.

MATERIALS AND METHODS

Fruits of *Litchi chinensis* Sonn. cv. Deshi were collected from Agriculture College, Sabour, Bhagalpur at the partially mature (45 days after anthesis), ripening initiation (52 days after anthesis), partially ripe (59 days after anthesis) and fully ripe (66 days after anthesis) stages. The ripe fruits were picked, surface sterilized with 1% chlorine-water and stored in polythene bags at 10-12°C. Harvested fruits were studied at overripe (73 days after anthesis), partially senescent (80 days after anthesis) and senescent (87 days after anthesis) stages.

COLORIMETRIC ESTIMATION

A weighed amount of aril tissue was homogenised in water and TCA-precipitated protein was dissolved in NaOH and assayed colorimetrically (Lowry *et al.*, 1951) employing bovine serum albumin (BSA) standards.

ELECTROPHORETIC ANALYSIS

Soluble proteins were extracted by grinding the sample (250 mg/ml) in 0.2M Tris-HCl buffer (pH 8.0), 50 mM mercaptoethanol, 5 mM EDTA and 1% polyvinyl pyrrolidons (PVP). The mixture was centrifuged and the clear supernatant (0.1 ml, equivalent to 25 mg tissue) was used for polyacrylamide gel electrophoresis. Preparation of gel columns, running of the gels on 7% acrylamide and staining of separated protein bands in Coomassie blue were followed according to Smith (1976). Bromophenol blue (BPB, 1% in 40% sucrose solution) was used as a tracking dye and BSA as a standard protein. Current at 220 volts was initially maintained at 1 mA per tube which was later increased to 3 mA per tube.

The relative mobilities (R_f) of protein bands were calculated using BPB as reference ($R_m = 1.0$). The gel columns were scanned in a densitometer (Type CM 11, Toshniwal make). The concentration of each band was calculated with the help of the band area of BSA of standard concentration and expressed as $\mu\text{g protein/mg tissue}$ on fresh weight basis. Data at each stage are the average of six replicates (\pm standard error, S.E.).

In addition to variance analysis (one way classification), polynomial regression (third order) for both colorimetrically and electrophoretically determined protein values was carried out with days after anthesis according to Overall & Klett (1972).

RESULTS AND DISCUSSION

COLORIMETRIC STUDIES

From partially mature to ripening initiation

stage, the total protein content greatly declined followed by its rapid rise at partially ripe stage. After its rapid decline at fully ripe stage, it marginally increased at overripe stage followed by rapid rise in its content at partially senescent and senescent stages (Table 1, Fig. 1).

Rise in total protein at partially ripe stage (Table 1) in litchi is in agreement with that observed by Sah

et al. (1984) who correlated rise in protein due to *de novo* protein synthesis in the course of ripening of litchi. Studies on other fruits including banana (Brady & O'Connell, 1976) and tomato (Baker *et al.*, 1985) point to the role of protein synthesis during fruit ripening and suggest an increase in the rate of protein synthesis during the climacteric rise, followed by a decline later in ripening (Brady, 1987). In the present work on litchi, final phase of ripening was associated with high protein content presumably due to inhibited proteolysis, influenced possibly by storage of fruits in polythene bags stored at 10-12°C.

ELECTROPHORETIC STUDIES

General distribution of protein components separable electrophoretically in the aril (Table 2, Fig. 2) displays a characteristic staining pattern containing protein bands of widely different charge and size at each stage of ripening and senescence studied. Relative concentration of protein bands at various stages fluctuated (Table 2). On the basis of R_m , five electrophoretically separable protein bands in aril of litchi cv. Deshi were detected in course of fruit ripening and senescence, although not more than two bands were detectable at any stage studied (Fig. 2). Such changes in protein patterns in course of fruit ripening have been reported in other fruits (Hobson, 1974; Biggs *et al.*, 1986). The cause of ripening initiation is not clear but such evidences suggest that the main event during fruit ripening results from a sequential synthesis of specific proteins which may be crucial for the ripening phenomenon (Hobson, 1974). Patterns of tomato polypeptides translated *in vitro* in response to mRNAs from early stages of fruit development differ from those translated in response to mRNAs from later stages of fruit development (Slater *et al.*, 1985). Such protein patterns are attributable to the protein breakdown and reassociation of units which play an important role in the understanding of fruit senescence, as in leaves and petals (Parups, 1971). However, this is far from understood at present due to lack of conclusive information.

A noteworthy point is that changes in colorimetrically and electrophoretically determined proteins do not correspond with each other at some stages studied. This suggests that changes in soluble proteins are independent of changes in structural proteins. Regression curves for protein values in the aril in both colorimetric and electrophoretic investigations are

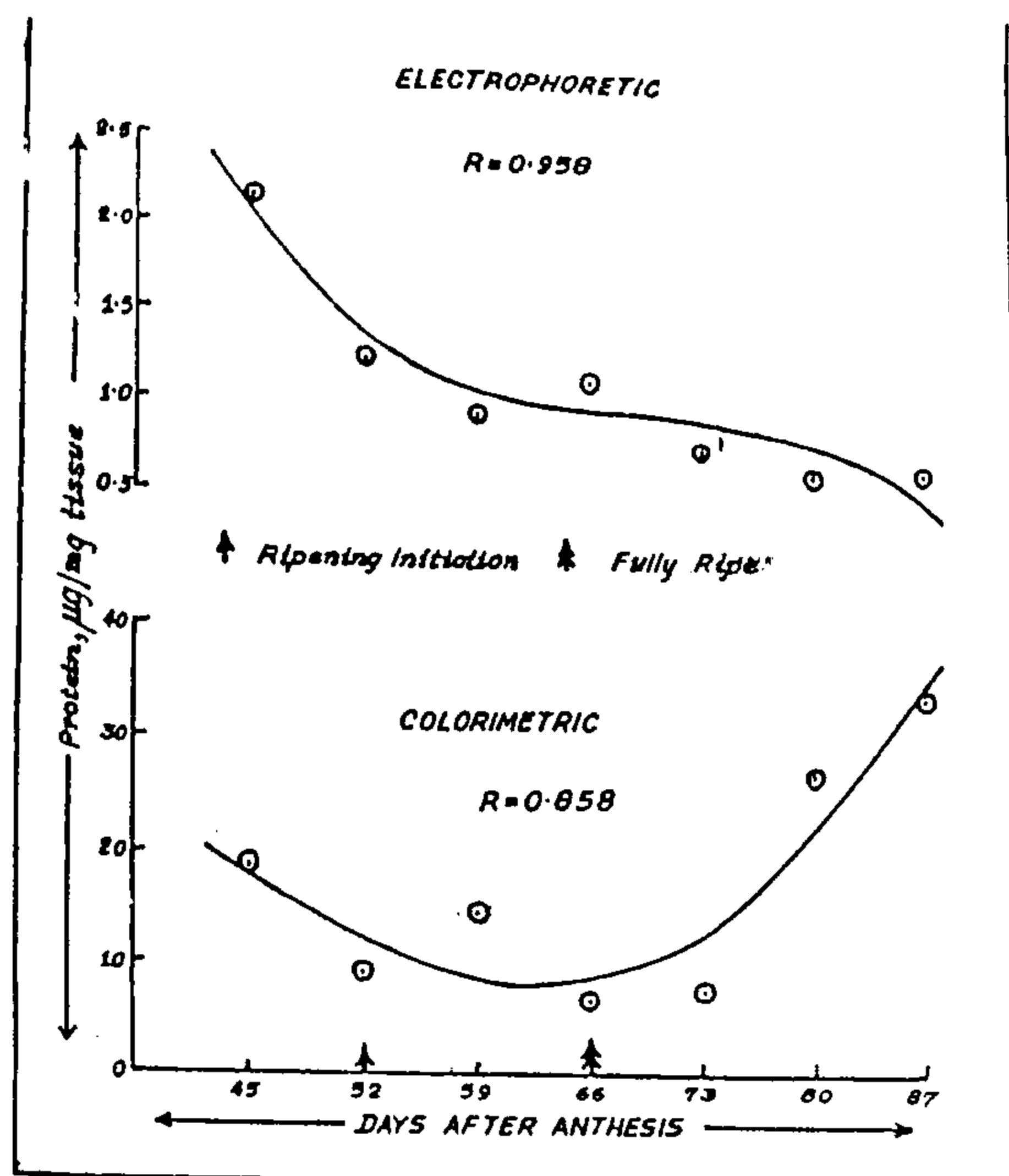


Fig. 1. Regression curve of protein content in aril of litchi cv. Deshi during ripening and senescence according to equation, $Y = a_0 + a_1D + a_2D^2 + a_3D^3$ (where Y = protein amount, D = days after anthesis and a_0, a_1, a_2 & a_3 = coefficients of regression; for electrophoretic analysis, $a_0 = 22.63$, $a_1 = -0.92$, $a_2 = 1.31 \times 10^{-2}$ & $a_3 = -6.24 \times 10^{-3}$, whereas for colorimetric analysis, $a_0 = 65.75$, $a_1 = -0.40$, $a_2 = -3.12 \times 10^{-2}$ & $a_3 = 1.31 \times 10^{-3}$).

Table 1: Protein content (colorimetric) in aril of litchi cv. Deshi during ripening and senescence.

Stages of fruit development	Fruit wt., g.	Days after anthesis	$\mu\text{g Protein}/\text{mg tissue} \pm \text{S.E.}$
I. Partially mature	3.16	45	18.37 \pm 2.15
II. Ripening initiation	7.03	52	8.90 \pm 0.64
III. Partially ripe	11.65	59	14.71 \pm 1.45
IV. Fully ripe	13.12	66	6.49 \pm 0.61
V. Over ripe	12.45	73	7.27 \pm 0.94
VI. Partially senescent	10.40	80	27.72 \pm 2.62
VII. Senescent	10.65	87	34.08 \pm 0.81

C.D at 5% level of significance = 4.31

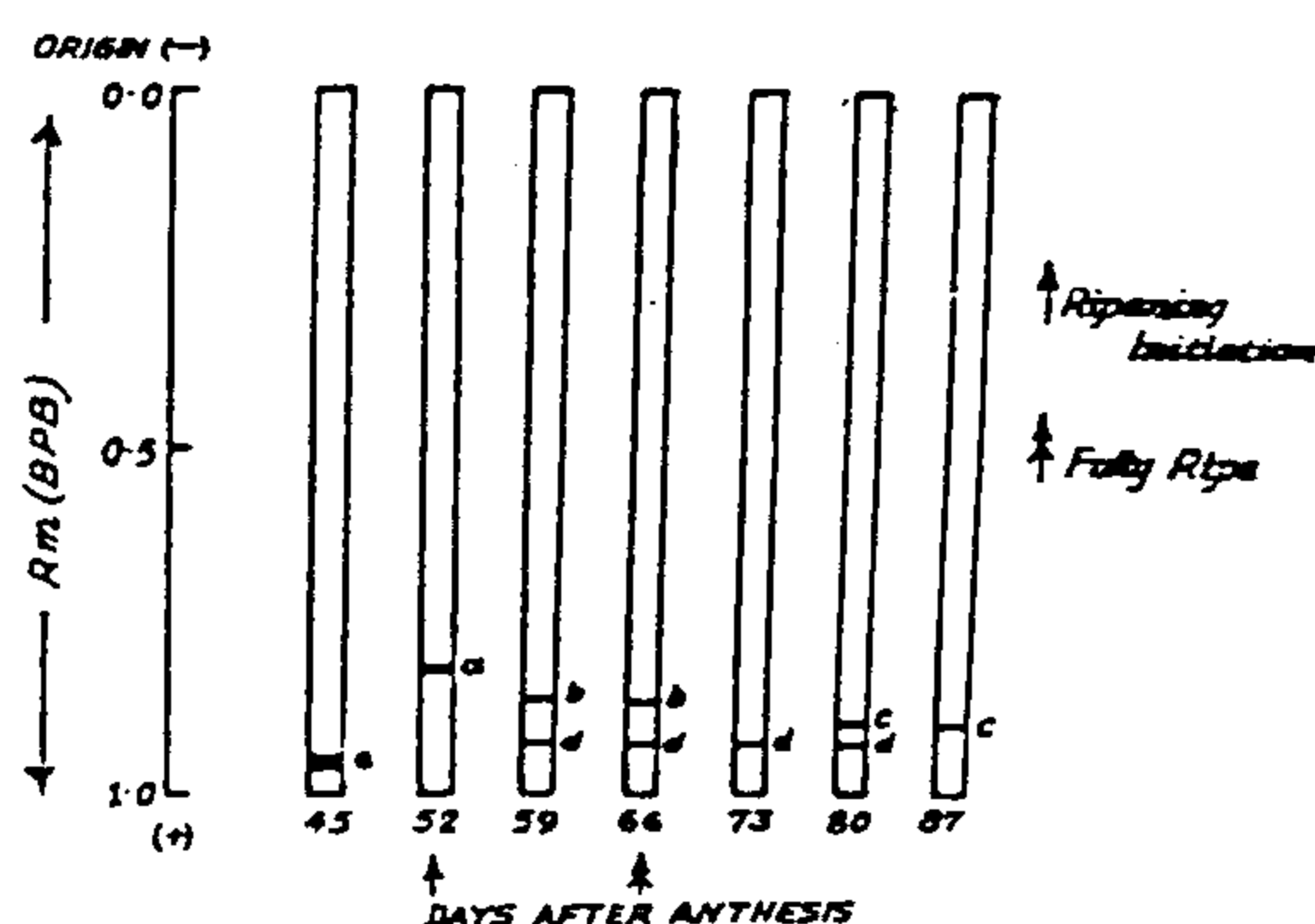


Fig. 2 Electrophoregram of protein in aril of litchi cv. Deshi during ripening and senescence.

Table 2: Content of protein profiles (electrophoretic) in aril of litchi cv. Deshi during ripening and senescence.

Stage of fruit development	Concentration of protein bands, μg protein /mg tissue					Total \pm S.E.
	'a'	'b'	'c'	'd'	'e'	
	Rm	Rm	Rm	Rm	Rm	
	0.82	0.87	0.90	0.93	0.96	
Partially mature	-	-	-	-	2.13	2.13 \pm 0.39
Ripening initiation	1.23	-	-	-	-	1.23 \pm 0.32
Partially ripe	-	0.53	-	0.40	-	0.93 \pm 0.15
Fully ripe	-	0.50	-	0.63	-	1.13 \pm 0.08
Over ripe	-	-	-	0.74	-	0.74 \pm 0.18
Partially Senescent	-	-	0.36	0.27	-	0.63 \pm 0.12
Senescent	-	-	0.66	-	-	0.66 \pm 0.22

C.D at 5% level of significance = 0.67
(Rm = Relative mobility)

also different (Fig. 1). As the value of R were very close to 1.0 in both colorimetrically (0.858) and electrophoretically (0.958) analysed proteins, their contents can be calculated for each days of fruit ripening and senescence in the aril with the help of regression curves. Such theoretically predictable values came fairly close to experimentally obtained ones in the present study which tend to confirm the view that ripening is a phenomenon in fruit ontogeny reflecting the expression of a discrete genetic message.

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