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Research on the Utilisation of the Pigment from '*Phytolacca decandra* L.' As a Food Colourant: Part 2—Tests on Pigmenting Power and Stability of Phytolaccanin in Model Solutions*

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ABSTRACT

Some tests on the colouring power and stability of the colourant extracted from Phytolacca decandra *L. are described.*

The colourant, which appears just perceptibly pink at 0·5 ppm, magenta at 5 ppm and dull red-purple at 30 ppm, can be used in foods with a wide range of acidity since it is not influenced by pH in technical grade purification.

However, owing to its poor stability at room temperature, the colourant is proposed for refrigerated and frozen foods or for foods where betanin (identical compound) is permitted.

INTRODUCTION

In a previous paper (Forni *et al.*, 1983), we described the preparation of a pigment concentrate from *Phytolacca decandra* berries freed from toxic saponins by precipitating with concentrated H_2SO_4 and further purified by partitioning with butanol and ethyl ether. The product contained 12% of Phytolaccanin pigment on a dry matter basis, together with 50% of sugars, polyphenols and ash.

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The very simple procedure did not require chromatographic or electrophoretic separations, which would be necessary to extract a highly purified compound. The first necessary qualification of a food colourant is that it must be harmless, although not necessarily pure. The effect of other components present in the pigment concentrate on the behaviour of the colourant was examined in comparison with highly purified Phytolaccanin added as a food colourant. Von Elbe *et al.* (1974) found better betanin stability when it was in the form of a concentrated red beet juice than when it was used as a pure compound, ascribing this to a protective effect of other constituents of the juice. The pigmenting power and stability of the concentrate added to model systems and the protective effect of ascorbic acid were also investigated.

MATERIALS AND METHODS

Preparation of the technical Phytolaccanin (TP)

This was done according to the procedure reported in the previous paper (Forni *et al.*, 1983) which can be summarized as follows. The pokeweed berries were extracted at room temperature for 30 min with double their weight of acidified water (pH 4·5, citric acid); after centrifugation the solution was cooled to 5 °C, with the addition of H_2SO_4 ($d = 1{\cdot}84$) to reach pH = 1. After 5 min it was again centrifuged to remove the precipitated saponins, then treated with $Ba(OH)_2$ solution to pH = 5. After the removal of precipitated $BaSO_4$ by centrifugation, the pigment solution was partitioned three times with 1-butanol and once with diethyl ether. The pigment solution was evaporated to a 70° Brix concentration, then stored in a refrigerator (5 °C) in the dark. This concentrate contained 12·9% of Phytolaccanin, on a dry matter basis.

Preparation of the purified Phytolaccanin (PP)

The method of Von Elbe *et al.* (1972) for the extraction of the betanin from red table beets was adopted for *Phytolacca decandra* berries. The berries were pressed lightly to avoid rupturing the seeds, then they were extracted by agitating the pressed juice for 30 min with water acidified with citric acid at pH 4·5 in a volume to volume ratio of 1:25.

Two hundred millilitres of the extract were loaded on the top of a column, 2 × 10 cm, filled with Dowex 50 W–X2, previously conditioned with water followed by 0·1N HCl, according to the method of Piattelli & Minale (1964). After removing sugars and other soluble substances from the column by 0·1N HCl, the pigment was eluted by water then concentrated to 10 ml at 45 °C in a vacuum in a rotary evaporator.

The pigment solution was chromatographed on a column of insoluble PVP (Polyclar AT), 5 × 30 cm, preconditioned with water. The fractions eluted with water were collected in a fraction collector. Magenta coloured fractions with maximum absorption at 538 nm were pooled, concentrated under vacuum and again chromatographed on insoluble PVP.

The procedure was repeated twice. The pigment was finally freeze-dried, then stored at −30 °C under vacuum. The purity test results agreed with those quoted by Wyler & Dreiding (1961).

Tests on the utilisation of the pigment

(*1*) *Colour power*

The chromatic characteristics of the pigment were evaluated in aqueous solutions: (a) according to the different concentrations of Phytolaccanin at pH = 5 from 0·5 ppm to 30 ppm and (b) at the constant concentration of 15 ppm, varying pH from 3 to 8.

Chromatic data were taken either subjectively with a Munsell chart or objectively with a Hunterlab D25D colorimeter.

The optical density was taken at 538 nm.

(*2*) *Evaluation of the colourant stability in a model solution*

Tests were carried out by adding the colourant to a model solution simulating a base for both a refreshing alcoholic drink and a soft beverage of the following composition: sucrose, 160 g litre^{-1}; glycerol, 10 g litre^{-1}; tartaric acid, 3 g litre^{-1}; citric acid, 1 g litre^{-1}; acetic acid, 0·5 g litre^{-1} sodium chloride, 0·5 g/litre^{-1} and sodium fluoride, 1 g litre^{-1} (antiseptic additive). For the alcoholic drink, 17% ethanol (EtOH) was added. The protective action of 1 g litre^{-1} ascorbic acid (AA) was also tested.

The colour of the beverage was selected by a panel of ten judges and corresponded to an addition of 20 ppm of the pure pigment.

(*2(a)*) *Influence of the purification grade of the pigment:* Tests were carried out to compare the model beverage (MB) coloured with the

purified pigment (PP) with the technical pigment (TP) at the same chromatic values at 20 °C as follows:

Samples:
1P = MB pH 3 + PP
1T = MB pH 3 + PP
2P = MB pH 3 + EtOH + PP
2T = MB pH 3 + EtOH + TP
3P = MB pH 3 + EtOH + AA + PP
3T = MB pH 3 + EtOH + AA + TP

(*2(b)*) *Influence of storage conditions on the stability of the colourant in the model beverage:* The parameters evaluated were as follows.

Temperature: 4 °C, 25 °C and 40 °C.
Light: room light and darkness at 25 °C.
pH: pH 3 and pH 5 (citric acid).
Antioxidant addition: Ascorbic acid (AA) 1 g litre^{-1}.
Ethanol 17 % (EtOH) to evaluate the difference in stability between alcoholic and soft drinks

The solutions tested were as follows: 1 = MB pH 3; 2 = MB pH 3 + EtOH; 3 = MB pH 3 + EtOH + AA; 4 = MB pH 5; 5 = MB pH 5 + EtOH; 6 = MB pH 5 + EtOH + AA.

METHODS

The pigment degradation was studied in the solution over a period of time until total bleaching was achieved by measuring the absorbance (optical density) at 538 nm for the Phytolaccanin concentration, and that at 476 nm for the brown substances possibly formed against the blank solution (no pigment added). The chromatic change was evaluated by scanning the solution by absorption spectrophotometry (C. Erba Spectracomp 601) in the visible range and by calculating the CIE specifications by the weighted ordinate method (MacKinney & Little, 1962), processing spectrophotometry data by a programme expressly made in mini Basic Olivetti language for an Olivetti P6040 minicomputer.

Hunter units L, a and b (Hunter, 1975) were adopted as a colour scale; therefore, colour differences were computed as NBS units:

$$E = \pm\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

RESULTS

(*1*) *Colouring power*

Figure 1 illustrates the colour of pure Phytolaccanin aqueous solutions in concentrations increasing from 0·5 to 30 ppm plotted on the Hunter colour solid. It can be seen that the colour situated in a purple area started from a just perceptible pink (Munsell notation = 5RP/8/4), then increased in saturation and lightness, with a small change in hue, to magenta, with 9 ppm (5RP/6/10), next turning dull red-purple with 30 ppm (5RP/4/12). This colour is very strong and of adequate

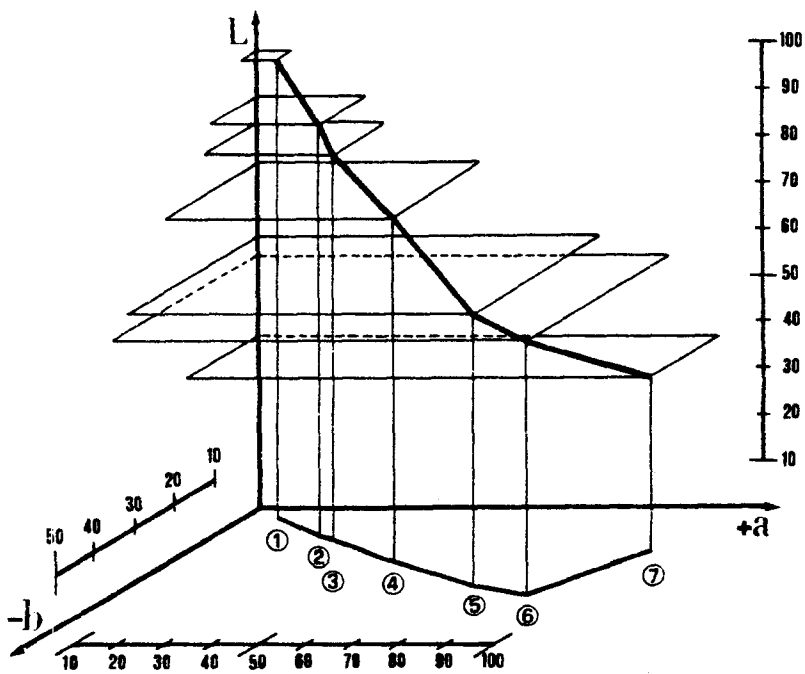

Fig. 1. Hunter colour solid of Phytolaccanin solutions. 1 = 0·5 ppm; 2 = 1·8 ppm; 3 = 2·5 ppm; 4 = 4·4 ppm; 5 = 8·9 ppm; 6 = 11·5 ppm; 7 = 30·6 ppm.

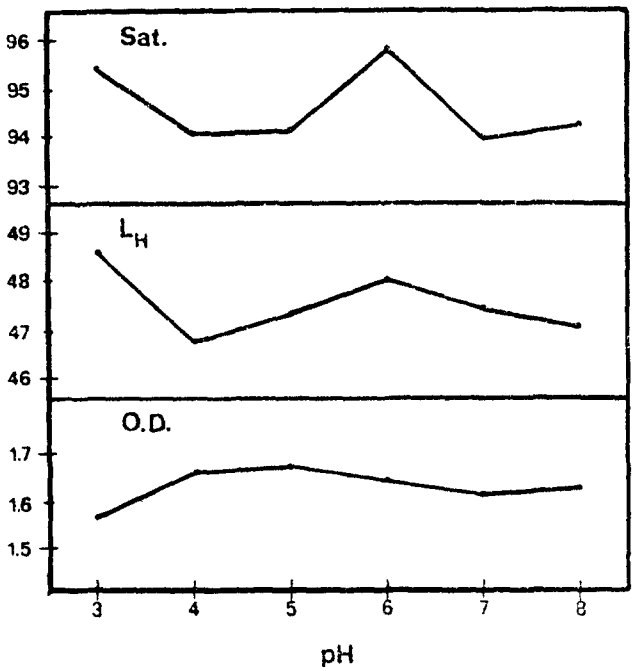

Fig. 2. Influence of pH on Phytolacannin colour.

acceptability for a beverage. The pH did not significantly influence either optical density or the chromatic characteristics even though there is a maximum of saturation at pH 6: therefore, the colouring matter can be added to food of different pH without notable discolorations (Fig. 2).

(*2*) *Stability of the colourant in the model solution*

(*2(a)*) *Influence of the purification grade:* The influence of the purification grade is summarized in Fig. 3 for both the percentage decay of the pigment and the chromatic changes during 60 days at 20 °C. It appears that, whilst there was no difference in stability during storage at 20 °C between the two purity grades for either a soft or an alcoholic base, adding ascorbic acid had a protective effect only on the technical grade product (sample 3T, Fig. 3(a)); the same effect could be observed on the Hunter chromaticity diagram (Figs 3(b) and 3(c)) as well as from the colour differences (Fig. 3(d)). Therefore, a synergistic effect of AA with the natural antioxidants present in the technical product might be assumed. After total bleaching, the solution with the addition of TP

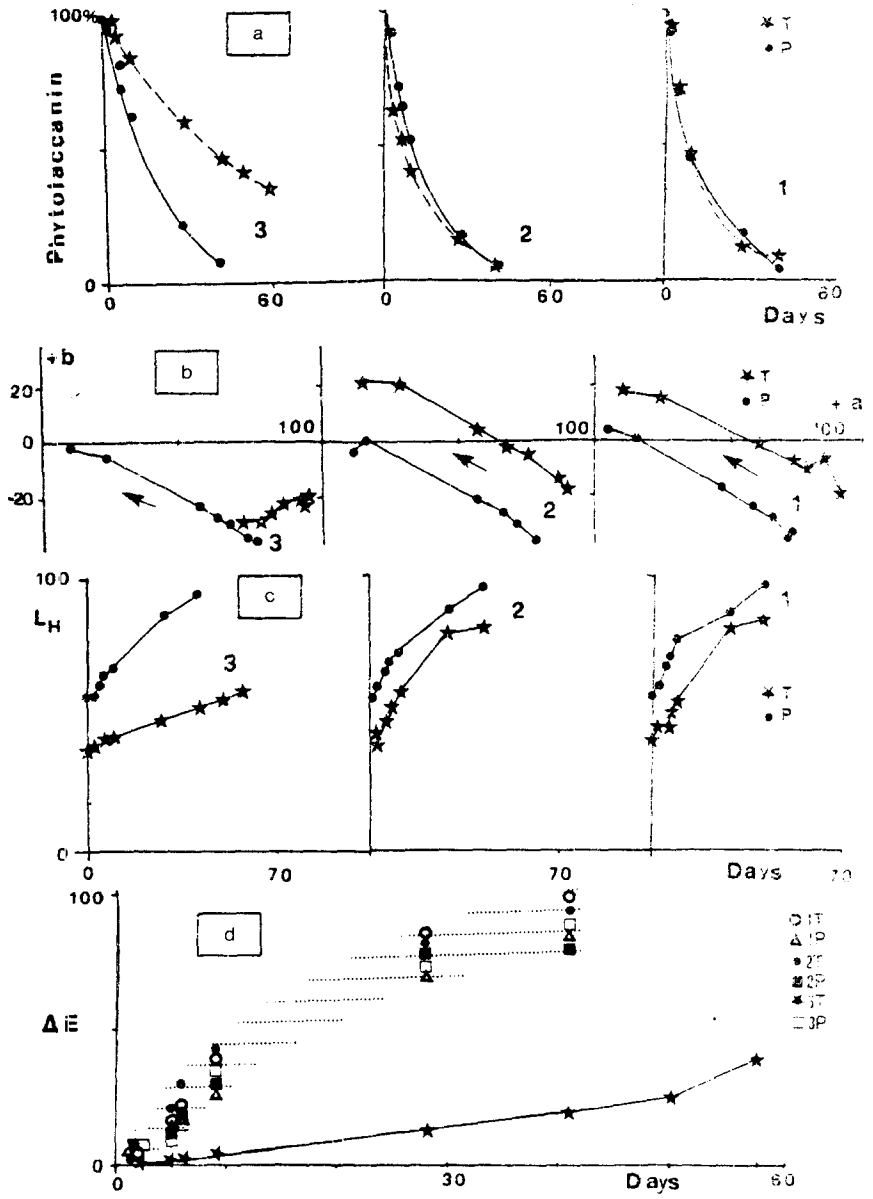

Fig. 3. Stability of the technical grade pigment as compared with the purified one. (a) Per cent decay; (b) Hunter *a*, *b* colour plane; (c) Hunter L_H values; (d) ΔE = colour differences; test samples: see text.

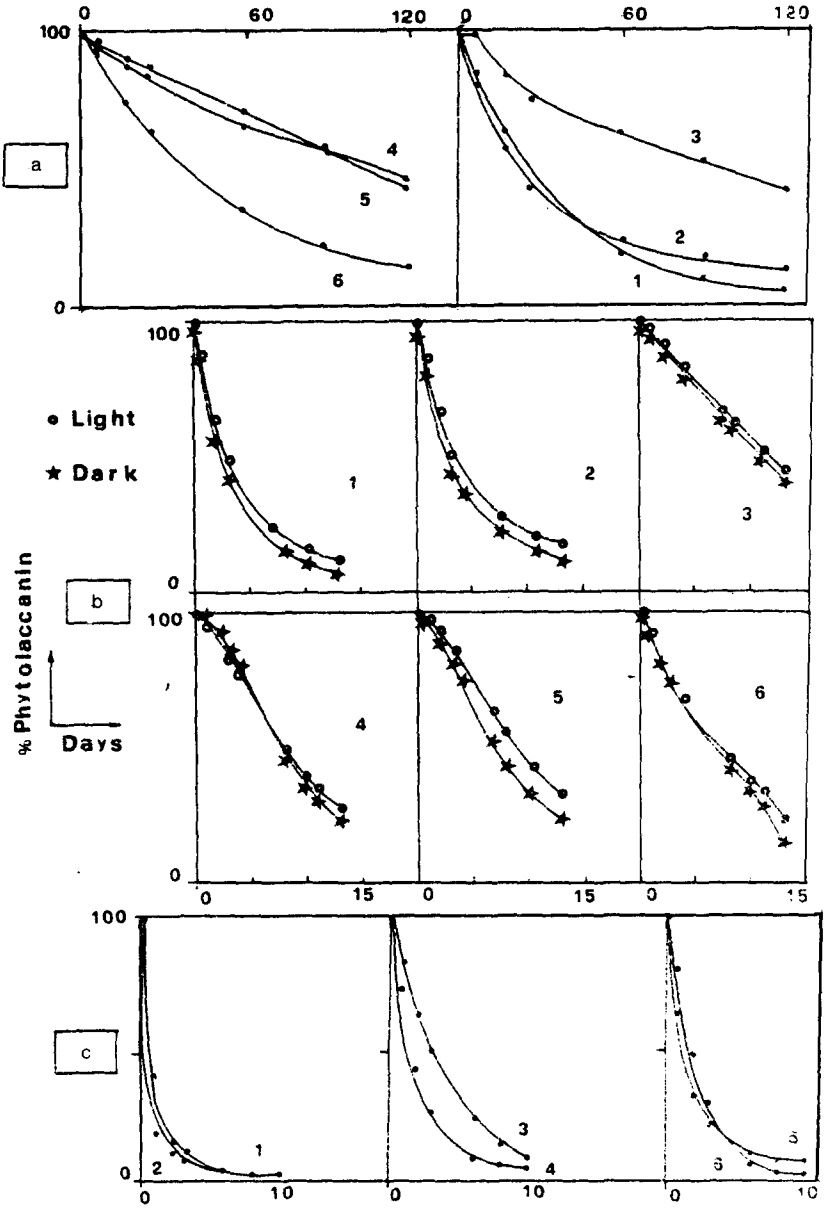

Fig. 4. Per cent decay of the Phytolaccanin in the model beverage (MB) during storage: (a) $t = 4\,°C$ dark; (b) $t = 25\,°C$, light and dark; (c) $t = 40\,°C$, dark; test samples: see text.

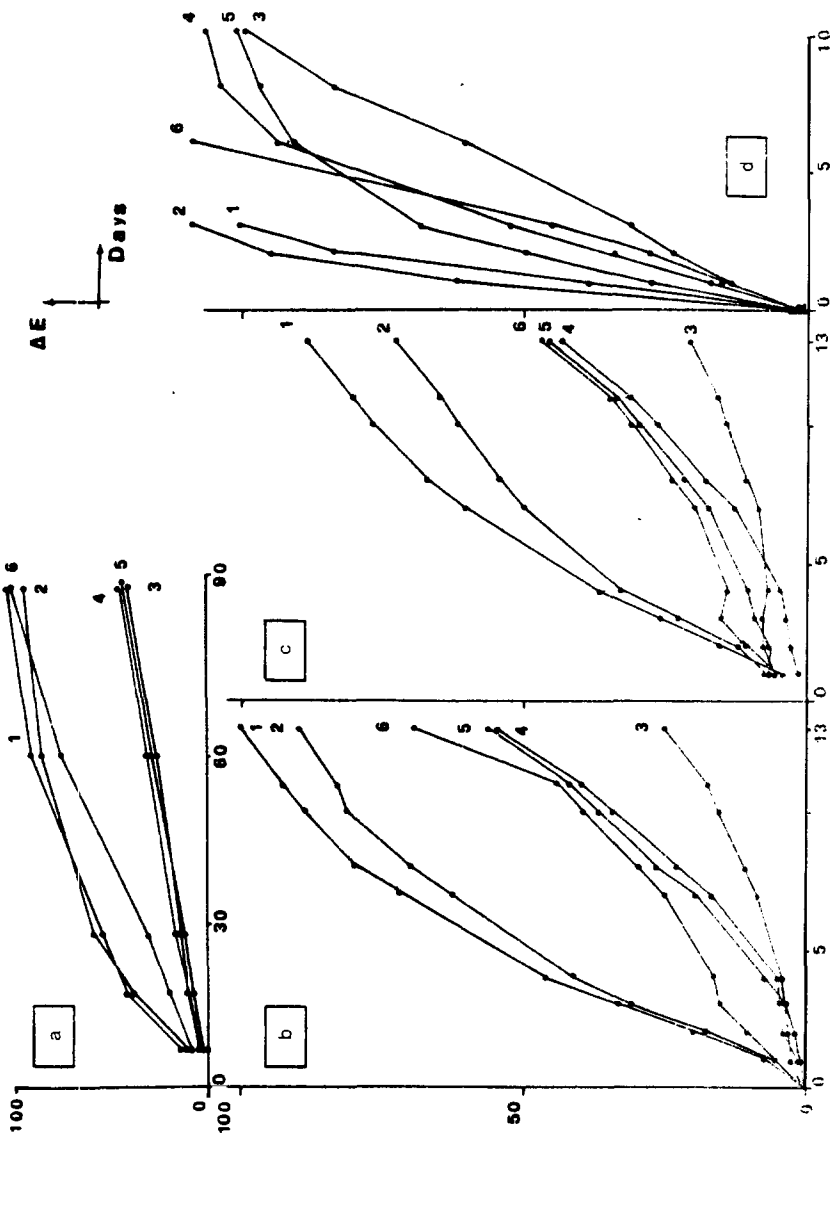

Fig. 5. Δt = Colour differences of Phytolaccanin in MB during storage: (a) $t = 4$ °C, dark; (b) $t = 25$ °C, light; (c) $t = 25$ °C, dark; (d) $t = 40$ °C.

appeared yellow, while that with PP was colourless, suggesting that some browning reactions had arisen due to impurities of the technical product.

(*2(b)*) *Influence of storage conditions on the stability of the colourant:* The effect of temperature on the pigment bleaching is very clear: at 4 °C the half shelf life could reach about 95–100 days (sample Nos. 3, 4 and 5, Fig. 4(a)), while at 25 °C only sample 3 reached 10 days (Fig. 4(b)). At 40 °C the half-life was only 3 days and the total bleaching was achieved within 10 days (Fig. 4(c)). No significant influence of light or dark was observed (Fig. 4(b)).

With regard to the effect of pH, it was seen that, at pH 3, the addition of ascorbic acid was helpful in extending the half shelf life of the colourant, whilst, at pH 5, adding AA accelerated bleaching (Fig. 4(a), sample Nos. 3 and 6). Therefore, it appeared that the addition of ascorbic acid as a protective agent is limited by the pH of the beverage. No effect of added ethanol was observed.

Similar behaviour was observed in the chromatic changes during storage. An acceptable colour difference could be maintained for 30 days at 4 °C but for no more than 5 days at room temperature (Figs 5(a), (b) and (d)).

DISCUSSION

The tests referred to above were completed by checking the influence of single components of the model solution on the stability and chromatic characteristics of the pure colourant at 20 °C. Only the most significant results are reported here (Fig. 6) where a slight favourable effect of the alcohol was evident, while pH = 5 resulted in the best stability, agreeing with results ascertained by Von Elbe *et al.* (1974) for betanin. The effect of the ascorbic acid on the hue of the solution is also shown (Fig. 6(b)): during the first days of storage the colour turned purple before starting to bleach.

From these results it appears that the Phytolaccanin can safely be used as a food colourant in a technical grade of purification such as that obtained by the method described in the previous paper (Forni *et al.*, 1983). As the colour is not influenced by pH, it can be used in a wide range of foods of varying acidity. However, there are some limitations due to its insufficient stability at room temperature. Adding ascorbic acid can help

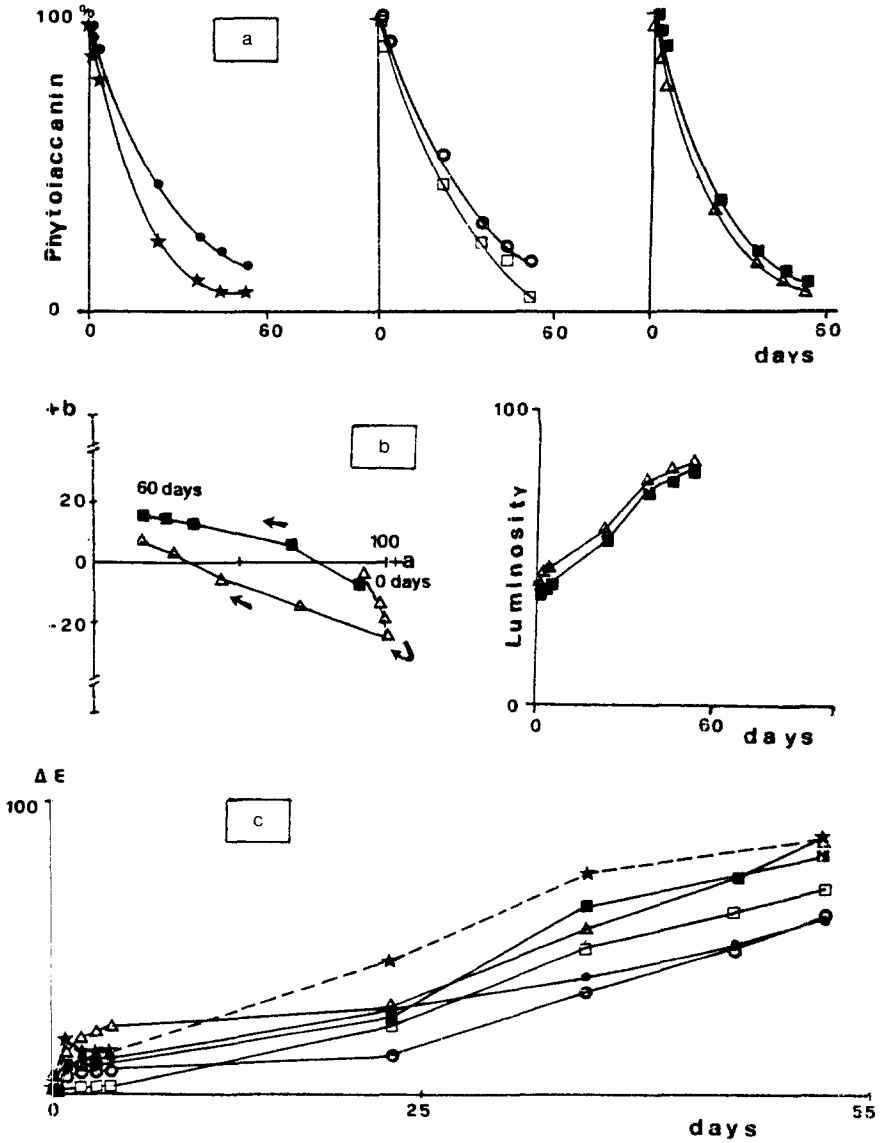

Fig. 6. Influence of some MB single components on Phytolaccanin stability: (a) Per cent decay (OD); (b) Hunter L, a and b values for citric acid and ascorbic acid; (c) ΔE = colour differences.

★ pH 3. □ Sucrose. □ Citric acid.
○ pH 5. ○ Ethanol. △ Ascorbic acid.

to extend the half shelf life only for beverages at pH 3; the best way to increase this period of time is by storing at low temperature. Therefore the colour can be recommended for refrigerated and frozen food. However, it can substitute for betanin in colouring foods where this substance is allowed by law as it is, in fact, the same chemical compound, with the added advantage of very easy extraction and purification. The similarity of behaviour of the two colourants has already been ascertained by Driver & Francis (1979) who have compared the stability of Phytolaccanin and betanin in dessert gels.

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