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Research Article

The effect of light, temperature, ph on stability of anthocyanin pigments in *Musa acuminata* bract

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The anthocyanin pigment was extracted from *Musa acuminata* bract using the acidified methanol. The extracted anthocyanin pigments then were exposed to number of environmental conditions, which could destabilize the anthocyanin molecules. These environmental conditions were included fourteen different pHs, various temperatures and presence or absence of light. The temperature stability of the anthocyanin extract was calculated by reaction velocity constants (k) as well as the half-life time (t1/2). The results of the study showed that increasing in pH, temperature or exposure to light is able to spoil the anthocyanin molecule. Copigmentation of anthocyanin resulting in increase in both hypochromic effects and bathochromic shifts. *Musa acuminata* bract anthocyanin extract was more stable at pH 5.1 and 6.0, temperature at 20˚ C and 30˚ C both in the presence and absence of light. This characteristic differs from other anthocyanins. This property could facilitate its application as a natural food colorant.

Keywords: *Musa acuminata* bract, anthocyanin, Color stability, pH, temperature, light.

Anthocyanins are a group of reddish-blue, water-soluble pigments common in many flowers, fruits and vegetables and they can be included in the category of natural additives (Francis *et.al.*, 1989). The interest of the food industry in natural colorants replacing synthetic dyes has increased significantly over the decades, mainly due to safety issues (Garcia-Falcon *et.al.*, 2007). Although anthocyanins are less stable in various environmental condition, they include varieties of colors such as orange, red, maroon and blue which make them an attractive alternative as coloring agents in food industries (Markakis, 1982; Francis, 1989). The intensity and stability of the anthocyanin pigments is dependent on various factors including structure and concentration of the pigments, pH, temperature, light intensity, quality and presence of other pigments together, metal ions, enzymes, oxygen, ascorbic acid, sugar and sugar metabolites, sulfur oxide etc. (Mazz and Minitiati, 1993; Francis, 1989). Anthocyanins have four different structures, which are in equilibrium and include flavylium cation, quinoidal base, carbinol pseudobase and chalcon. The relative amounts of these structures in equilibrium are varied and depend on the pH and anthocyanin structure (Mazz and Minitiati 1993).

Another factor in increasing the stability of the anthocyanin is the co-pigmentation (Francis, 1989; Malien-Aubert *et al*., 2001). There are also more stables color pigments exist in the fruits and vegetables, which their phyto-chemical structure and anthocyanin properties need to be investigated. Recent studies using purified anthocyanins or anthocyanin rich extracts in *in vitro* experimental systems have confirmed the coloring potential of these pigments. According to Delgado-Vargas *et al*. (2000), the commercial production of natural pigments has been retarded due to shortage of significant quantities of highly pigmented fresh plant tissues and lack of simple and efficient methods of extraction and purification of these products from plants. However, the replacement of synthetic dyes with natural pigments presents problems including lack of pigment sources that can be used commercially, low stability and lack of simple and efficient methods of extraction and purification of these products (Ozela, 2004).

The stability of anthocyanins and the rate of degradation are notably influenced by temperature. Thermal stability of anthocyanins varies with temperature and pH. The presence of oxygen and interactions with other components, like sugars and ascorbic acid also affect anthocyanin stability. The main cause of pigment color loss seems to be related to anthocyanin hydrolysis due to the observed proportionality between the speed of red color disappearance from anthocyanins and the velocity of free sugar formation. Heat causes anthocyanins, which are found at pH 2.0 to 4.0, to undergo hydrolysis at glycoside linkages to produce chalcone and, later, alpha-diketones (Adams, 1973).

Based on observation of a few relatively simple anthocyanins *in vitro*, the following scheme is generally accepted (Brouillard, 1988): at a pH of approximately 3 or lower, the anthocyanin is orange or red and exists as a flavylium cation. As the pH is raised, kinetic and thermodynamic competition occurs between the hydration reaction of the flavylium cation and the proton transfer reactions related to the acidic hydroxyl groups of the aglycone. While the first reaction gives a colourless carbinol pseudo-base, which can undergo ring opening to a chalcone pseudo-base, the latter reactions give rise to quinonoidal bases. Further deprotonation of the quinonoidal bases can take place at pHs between 6 and 7 with the formation of purplish, resonance-stabilised quinonoid anions. However, replacing synthetic dyes with natural colorants offers a challenge due to the higher stability of synthetic dyes with respect to light, oxygen, temperature, and pH, among other factors.

The overall goal of this study was to characterize the stability of *Musa acuminata* bract anthocyanin extracts. Our approach involved determining: (1) the pH effect on chromaticity (including the alkaline region) and (2) the effects of acylation, pH, temperature, and light on anthocyanin stability through time.

Materials and methods

Sample collection – *Musa acuminata* bract were collected from the field of vellore in udmalpet, Tamilnadu state, India and stored in sealed polyethylene bags at -20˚C until extraction.

Extraction - 0.5 gm of *Musa acuminata* bract were treated with 10 ml acidified methanol. And the mixture was centrifuged at 10,000 rpm for 10 min and supernatant was taken for analysis (Lachman *et.,al* 2003)

Analytical study

Stability studies on temperature

The effect of temperature on colorant stability was done with samples inside capped glass vials covered with aluminium foil sealed with parafilm and kept at different

temperatures ranging from 0˚C, 10˚C, 20˚C, 30˚C, 40˚C, 50˚C, 60˚C, and 70˚C. The UV/Vis spectra were recorded for freshly made samples (''0''), after 1 h, 1 day, and then subsequently after 2, 3, 4, 5 and 6 days at 520 nm.

Stability studies on Light

Light effect on colorant stability was performed with samples inside capped glass vials sealed with parafilm and kept at different temperatures ranging from 0˚C, 10˚C, 20˚C, 30˚C, 40˚C, 50˚C, 60˚C, and 70˚C. The UV/Vis spectra were recorded for freshly made samples (''0''), after 1 h, 1 day, and then after 2, 3, 4, and 5 days at 520 nm. The colorant half-life (t1/2) was determined for samples exposed to light and temperature only.

Stability studies on pH

a) Buffer solutions and anthocyanin solubility

0.05M (2µg/ml) of *Musa acuminata* bract were dissolved in acidified methanol. Each pigment solution was then divided into 14 equal portions, dried, and dissolved in appropriate volumes of buffers. The anthocyanin solutions were similarly prepared. All solutions were sealed, and kept at 10 °C during storage. The solvents used for preparations of the buffer-solutions were 0.2MKCl (A), 0.2 M HCl (B), 0.1 M $KHC_8O_4H_4$ (C), 0.1 M HCl (D), 0.1 M NaOH (E), 0.1 M KH_2PO_4 (F), 0.025 M borax (G), 0.05 M Na_2HPO_4 (H), and Table 1 shows the solvent proportions. The accurate pH-values were measured with a pH-meter .The pH values of the various samples did not change during storage.

b) Colour measurements

UV/Vis absorption spectra were recorded at 520nm for anthocyanin solutions at fourteen different pH-values (see Table 1) on a UV–Vis Spectrophotometer. As references, the respective buffer solutions were used. The UV/Vis spectra were recorded for freshly made samples (''0''), after 1 h, 1 day, and then after 2, 3, 4, 5, 6,13 and 20 days. The samples were kept in a refrigerator (25^{o}C) between the measurements.

Table 1 Colour stability solvent proportions (v/v) used in the buffer solutions

S.No	pH	A	B	C	D	E	F	G	H
1	1.1	27.17	72.83						
2	3.0			69.16	30.84				
3	4.1			99.80	2.0				
4	5.1			68.87		31.13			
5	6.0					10.07	89.93		
6	6.6					24.70	75.30		
7	6.8					30.94	69.06		
8	6.9					36.79	63.21		
9	7.2					40.97	59.03		
10	7.3					43.88			
11	8.0				29.08		56.12		
12	8.9				8.42				
13	9.9					26.79		73.21	
14	10.5					7.58			92.42

A-0.2M KCl ,B-0.2M HCl , C-0.1M $KHC_8O_4H_4$,D-0.1MHcl,E-0.1M NaOH,
F-0.1M KH_2PO_4,G-0.025M Borax,H-0.05M Na_2HPO_4

Results and discussion

Stability of anthocyanin from *Musa acuminata* bract at various parameters - In our experiments, the anthocyanin stability was optimized using various temperature, pH and light. The effects of light, temperature, and pH on the stability of anthocyanins were studied by several authors, and relationships between these effects and the decomposition of the anthocyanin pigments has always been observed (Stringheta, 1991; Kuskoski *et al*., 2000)

Spectrophotometric measurements of color intensity - Absorption spectra of *Musa acuminata* bract anthocyanin solutions were recorded using a UV-visible spectro-photometer. The change in the maximum absorbance (Amax) at varying wavelengths (λmax) presented the change in the color intensity, and revealed a possible hyperchromic effect (ΔAmax) and bathochromic shift (Δλmax), resulting from a copigmentation reaction.

The effect of temperature on the destruction of anthocyanin under light - Temperature is another factor, which has a role in destabilizing the anthocyanin molecular structure; with increase in temperature we see a greater degree of destruction in anthocyanin. In this research it has demonstrated that increasing time and temperature resulted in changes in anthocyanin and the copigmentation complex which resulted an increase in the visible spectrum (hyperchromic effect) and an increase in max (bathochromic shift) in the main peaks (Mohammad *et.al.,* 2006). These changes were shown in Figure 1.

The effect of eight different temperatures 0,10,20,30,40,50,60 and 70˚C on level of anthocyanin extracted from *Musa acuminata* bract during 5 days were measured at 520 nm. The results show that destruction of anthocyanin in 40, 50, 60 and 70˚C. There was an increase in absorbance of *Musa acuminata* bract anthocyanin which is stable at temperature 0, 10, 20˚C. We suggest that the speedy destruction of anthocyanin in higher temperatures could be due to hydrolyzation of 3-Glycoside structure, which has a protective effect in unstable anthocyanin. The other suggestion is that the hydrolyzation of the pyrilium ring resulted in production of chalcone, which are responsible for brown color developed food containing anthocyanin (Giusti and Wroslstad, 2000). Palamidis and Markakis (1975) has studied the effect of temperature on the stability of anthocyanin in soft drinks and have shown that increase in the storage temperature gradually accelerate the destruction of pigments in soft drinks. Spayd *et.al.*(2001) found that the increase in temperature accelerates the destruction of anthocyanins.

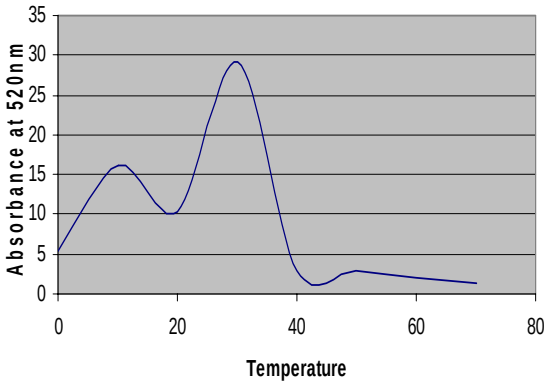

Figure 1 Stability of anthocyanins in various temperature (light)

In all reported research, a temperature increase in produce a decrease of the copigment bond intensity and its hyperchromic shift. Copigment complexes are exothermic and are particularly sensitive to temperature (Dangles and Brouillard, 1992). A temperature raise has been shown shift anthocyanin equilibrium towards the chalaone form, this has negligible effect in the visible range and the large decrease in absorbance with increasing temperature and

quantitative recovery of colour on cooling of copigment solution is due to changes the copigment complex (Brouillard *et.al.*, 1989).

The effect of temperature on the destruction of anthocyanin under dark - The effect of eight different temperatures 0, 10, 20, 30, 40, 50, 60 and 70˚C on level of anthocyanin extracted from *Musa acuminata* bract during 5 days were measured in separate instances. The results show that destruction of anthocyanin in 40˚C, 50˚C, 60˚C, and 70˚C. There was an increase in absorbance of *Musa acuminata* bract anthocyanin which is stable at temperature 10˚C, 20˚C and 30˚C **(Figure 2).** Timberlake (1989) suggested that light increase the flavylium cation construction, but in the absence of light the amount of chalcone in the extract containing anthocyanin was higher than its flavylium cation.

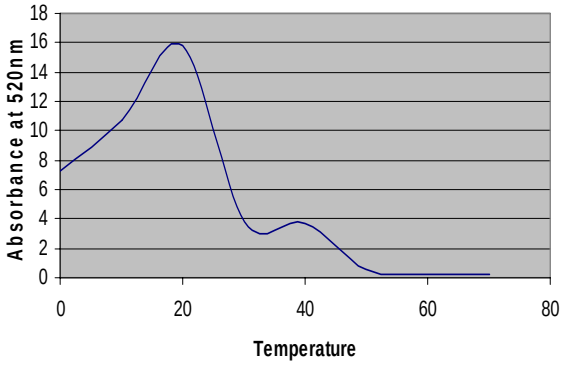

Figure 2 Stability of anthocyanins in various temperature (dark)

The effect of pH on the stability of anthocyanin - Another factor which affects the stability of anthocyanin is the pH. Our results showed that increasing pH cause greater destruction of anthocyanin in samples. Flavylium salts are stable only in highly acidic conditions. These salts loose the proton in higher pH and transform into quinoidal base, which is an unstable pigment, and immediately bond to water and form colourless compound called chromenol.

Colours of anthocyanins in freshly made samples at pH 1.1 to 10.5 - All the experiments were performed in a fixed temperature of 25ºC in an 20days period. The colour differences between the *Musa acuminata* bract anthocyanin vary with pH**.** These pigments had light pinkish at the lowest pH values. By stepwise pH increase until 7.3, the colour gradually changed toward more bluish tones. The aromatic acyl groups of purple corn clearly influenced the aglycone chromophore by inter intra-molecular association, creating more purple or bluish tones. (Kjell *et.al.*, 2004). *Musa acuminata* bract showed colourless structure at higher pH 10.5. At these pHs, the flavylium cation hydrated to yield the colourless carbinol (Mazza & Miniati, 1993).

Bathochromic shifts were observed for colourants at pH 1.1, 3, 4.1 and highest bathochromic shifts were observed at 6, 6.6. Increasing anthocyanin concentration, increase absorbance and the bathochromic shift occurs. *Musa acuminata* bract anthocyanin was stable at these pHs. The magnitude of increase in absorbance is depend on the copigment to anthocyanin ratio for a given anthocyanin (Mohammad *et.al.*, 2006**).**

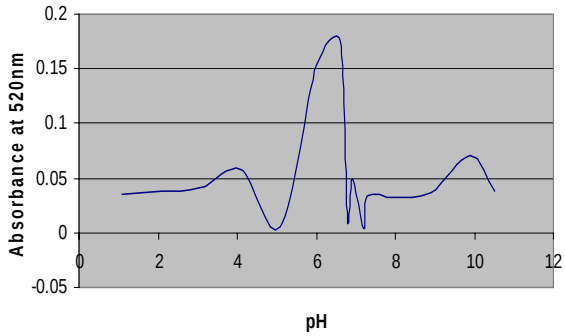

Figure 3 Stability of 0.05M crude Anthocyanins samples taken initially at various pH

Hypochromic shifts were observed for all colourants at pH 5.1, 7.2, 8, 8.9, 10.5. At higher pH, destruction of anthocyanin

occurs. Morris *et al*. (1986) have reported that in warm agricultural area, high pH of the grapes at the time of harvest could cause problem for the juice making industry. Higher pH in grapes can cause fading the colour and decrease in stability of the products. Angela and Little (1977) has studied the combination of the colour pigments in Strawberry jam and packaged Strawberries at the 37.7˚C during time. She has recorded the data for pH of 2, 3 and <1 and showed that destruction of anthocyanin pigments increases with increase in pH. (Figure 3)

Colour variation of anthocyanins in the pH range 1.1–10.5 after 1 hour dissolution

All the experiments were performed in a fixed temperature at 25^{o}C and after 1 hour incubation, the absorbance was recorded at 520nm. The same colour differences occurs between the *Musa acuminata* bract anthocyanin like colours of anthocyanins in freshly made samples. The visible absorption maxima for *Musa acuminata* bract suffered a bathochromic shift at pH 1.1. Hypochromic shifts were observed at higher pH 6, 6.6, 6.8, 6.9, 7.2, 7.3, 8, 8.9, 9.9. Red sweet potato extracts, at higher pH, showed hypochromic shifts with time related to chemical changes in the molecule (Inami, Tamura, Kikazuki, & Nakatani, 1996) due to interactions with the buffer used (Figure 4).

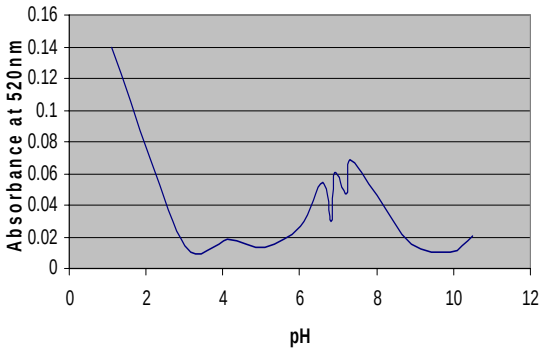

Figure 4 Stability of Anthocyanins samples taken after 1 hour at various pH

Colour variation of anthocyanins in the pH range 1.1–10.5 during storage - The colour differences between the *Musa acuminata* bract anthocyanin vary with pH. These pigments had light pinkish and yellow at the lowest pH values. *Musa acuminata* bract showed colourless structure at higher pH 10.5 In general, below pH 2, anthocyanins were primarily in the form of the red flavylium cation. When pH increased >2, there were rapid proton losses favouring red or blue quinonoidal forms. Through time, the flavylium cation became hydrated to yield the colourless carbinol or pseudobase, which equilibrated to the open chalcone form, also colourless (Mazza & Miniati, 1993).

Bathochromic shifts were observed at low pH. At pH values below about 1 for anthocyanin in aqueous solution, addition of copigment will reduce a bathochromic shift, but no increase in absorbance is observed in fact a small hypochromic shifts occurs (Mohammad *et al.,* 2006**).** Hypochromic shifts were observed at higher pH. A higher chroma at pH 7 for red sweet potato compared to purple corn was due to the presence of a purple-blue quinonoidal base with higher absorbance. Generally, this form is less stable and the absorbance decreases dramatically after a few minutes (Brouillard, 1982) (Figure 5).

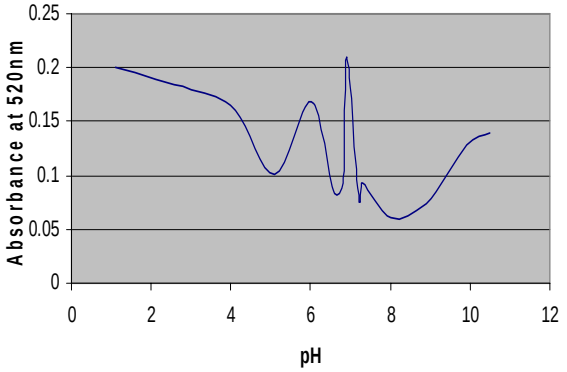

Figure 5 Stability of Anthocyanins samples taken after 20 days storage at various pH

Conclusion:

From the results it can be concluded that anthocyanin extracts of *Musa acuminata* bract were highly or moderately resistant to the pH, temperature and light factors tested. *Musa acuminata* bract anthocyanin extract was more stable at pH 5.1 and 6.0, temperature at 20˚ C and 30˚ C both in the presence and absence of light. Increase in environmental factors like pH, temperature and light accelerates destruction of anthocyanins. This studies verify our results.

According to Carvalho (1992), there is a need for replacement of the artificial dyes used in the food industry with natural dyes because of the general toxicity presented by artificial dyes, making them undesirable for human consumption. Thus these results suggest that anthocyanin extract from *Musa acuminata* bract represent inexpensive crops with high pigment yield that could be sources of anthocyanins for the food colorant market.

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References

Adams, J.B. 1973. Colour stability of read fruit. Food Manufacture **4**:19-20.

Angela, C. and Little, 1997. Colorimetry of anthocyanin pigmented products; Changes in pigment composition with time. J. Food Sci., **42:** 1570.

Brouillard, R. 1988. Flavonoids and flower colour. In The Flavonoids. Advances in Research Since 1980, ed. J. B. Har-borne, p. 525. Chapman and Hall, London.

Brouillard, R. 1982. Chemical structure of anthocyanins. p.1-40. In: P. Markakls (ed.) Anthocyanins as Food Colors. Academic Press, New York, USA

Brouillard, R., G.Mazza, Z. Saad, A.M. Albecht-Gary and A. Cheminat, 1989. The copigmentation re actions of anthocyanins: A microprobe for the structural study of aqueous solution. J. Am. Chem. Soc., **111**: 2604-2610.

Carvalho, P.R. 1992. Potencialidade dos conservantes naturais. Revista Brasileira de Corantes Naturais **4**:244-245.

Dangles, O. and R. Brouillard, 1992. A spectroscopic method based on the anthocyanin copigmentation interaction and appilied to the quantative study of molecular complexes. J. Chem. Soc. Perkin Trans.,**2**:247-257.

Delgado-Vargas, F.; A.R. Jiménez, and O. Paredes- López. 2000. Natural pigments: Carotenoids, anthocyanins, and betalains. Characteristics, biosynthesis, processing, and stability. Critical Reviews in Food Science and Nutrition. **40**:173-289.

Francis, F.J. 1989. Food colorants: anthocyanins, Crit. Rev. Food. Sci. Nutr., **28,** 273-314.

Giusti, M.M and R.E. Wrolstad, 2000. Characterization and measurement of anthocyanins by UV-visible spectroscopy. Food. Anal. Chem., Homepage.

Inami, O., Tamura, I., Kikazuki, H., & Nakatani, N. 1996. Stability of anthocyanins in Sambucus canadensis and Sambucus nigra. Journal of Agricultural and Food Chemistry, **44**, 3090–3096.

Kjell Torskangerpoll. and Oyvind M. Andersen, 2005. Colour stability of anthocyanin in aqueous solutions at various pH values. Journal of Food Chemistry, **89**, 427-440.

Kuskoski, E.M., P.T. Marques, and R. Fett. 2000. Estudo comparativo da estabilidade das antocianinas do baguaçu, jambolão e da uva. Revista Brasileira de Corante Natural (Brasil) **4:**73-76.

Lachman J., Orsák M., Pivec V. 2003. Antioxidant contents and composition in some vegetables and their role in human nutrition. *Zahradnictví – Hort. Sci.* (Prague), **27**: 65–78.

Garcia-Falcon, M.S., C. Perez-Lamela, E.Martinez-Carballo and J. Simal-Gandara, 2007. Food Chemistry , **105**, 248–259.

Malien-Aubert, C., O. Dangles and M.J. Amiot, 2001. Color stability of commercial anthocyanin – based extracts in relation to the phenolic composition. Protective effects by intra and intermolecular copigmentation. J. Agri. Food Chem., **49**: 170-176.

Markakis, P., 1982. Introduction In Anthocyanin in fruits, vegetables, and grain (pp. 1.28) Boca Roton,FL: CRC Press (Chapter 1).

Mazza, G. and E. Minitiati, 1993.Introduction. In Anthocyanin in Fruits, vegetables, and grains (pp: 1-28). Boca raton, FL: CRC Press(Chapter 1).

Mohammad Abyari, Reza Heidari amd Rashid Jamei, 2006. The effect of Heating, UV Irradiation and pH on Stability of Siahe Sardasht Grape Anthocyanin-copigment Complex. Journal of Biological Sciences, **6**(4), 638-645.

Morris, J.R. W.A. Sistrunk, J. Junek and C.A. Sims, 1986. Effects of maturity, juice storage, and juice extraction and temperature on quality on concord grape juice. J. Am. Sci., **111:** 742-746.

Ozela, E. F. 2004. Caracterização de flavonóides e estabilidade de pigmentos de frutos de Bertalha (*Basella rubra,* L.). Tese Doutorado em Ciência e Tecnologia de Alimentos. Universidade Federal de Viçosa. Viçosa, MG, Brasil. 71 pp.

Palamidis, N. and T. Markakis, 1975. Structure of anthocyanin. Food Sci., **40**: 104.

Spayd, S.E., J.M. Tarara, D.L. Mee and J.C.Feguson, 2002. Seperation of sunlight and temperature effects on the composition of *Vitis vinifera.* J. Food Sci., **53**: 3.

Stringheta, P.C 1991. Identifi cação da estrutura e estudo da estabilidade das antocianinas extraídas da infl orescência de capim gordura (*Mellinis minutifl ora.* Pal de Beauv), Campinas: Unicamp. Tese Doutorado em Ciência e Tecnologia de Alimentos. Faculdade de Engenharia de Alimentos, Universidade de Campinas., SP- Brasil, 138 pp.

Timberlake, C., 1989. Plant pigments for coloring food. J.B.N.F. Nutr., **14:** 113-117.