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Lipofuscin like compound in mango

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Abstract. Thin layer chromatographic separation of chloroform-methanol extracts of mango on silica gel revealed a fluorescent substance in mango peel and pulp. The compound had fluorescence spectrum similar to that of lipofuscin, the age pigment of animal tissues and was found to be water insoluble and stable to ultraviolet irradiation. The fluorescent material appeared to be a lipoprotein.

Keywords. Mango; lipofuscin; spectral characterization.

Introduction

Lipofuscin (age pigment) is known to accumulate in animal tissues during ageing and is extensively documented in literature on mammals (Reichel, 1971; Reddy *et al.,* 1973; Tauna, 1975). It is a compound formed by the complexation of protein with malonaldehyde derived by the peroxidation of poly-unsaturated lipids of subcellular membranes and is implicated in cellular damage due to lipid peroxidation and ageing (Hendley *et al.,* 1963; Porta and Hartraft, 1969; Herman, 1972). A lipofuscin like compound with typical fluorescent properties was reported in ripening pear and banana (Fletcher, 1973; Maguire and Haard, 1976). Recently a compound with fluorescent properties similar to lipofuscin was reported to be present in apples (Knee, 1982). It was of interest to see if such a compound accumulates in other fruits and we report the presence of a similar compound in mango peel and pulp.

Materials and methods

Fully ripe badami variety of mangoes were selected for this study. Freeze dried mango peel and pulp (5 g) respectively were extracted for 10 min at 50°C in 30 ml of chloroform-methanol mixture (2:1 v/v). The extract was filtered and evaporated to dryness under suction. The residue was dissolved in 3 ml of the chloroform-methanol mixture. An aliquot of this (0.3 ml) was spotted on a silica gel thin layer chromatography plate and developed with petroleum ether for 10 min. The plates were removed and were developed again with a mixture of petroleum ether—chloroform—methanol (3:1:1 — v/v) for a further period of 10 min. TLC

Abbreviations used: TLC, thin layer chromatography; UV, ultraviolet.

plates were exposed to ultraviolet light. The area on TLC plate at the origin containing the fluorescent compound was scrapped off the plate and the compound was extracted with chloroform-methanol (2 : 1 v/v). The extract was completely colourless and showed bright fluorescence when exposed to UV light. This was used for further studies. The fluorescence spectrum was recorded in a Perkin Elmer spectrophotofluorimeter. Quinine sulphate 0.1 µg in 0.1 N H_2SO_4, was used as a reference standard. The standard had a relative fluorescence intensity of 2.7. Both the excitation and emission spectra were obtained for the samples.

Lipid peroxidation was studied by the thiobarbituric acid method (Sidwell *et al.,* 1955; Holland, 1971). Protein (Lowry *et al.,* 1951), polyphenol (Swain *et al.,* 1959) and carotenoid estimations (Association of Vitamin Chemists, 1966) were carried out by the procedures indicated. Amino acids were separated by ascending paper chromatography on Whatman No. 2 paper using the following solvents (1) phenol-water-NH_3, 80 : 20 : 0.5 (v/v) (2) *n*-butanol-pyridine-water, 1 : 1 : 1 (v/v) and (3) *n* -butanol-aceticacid-water, 4 : 1 : 1 (v/v). The chromatograms were dried in air and the amino acids located by spraying with 0.5% ninhydrin in acetone and heating for 10 min at 60°C.

Results and discussion

In the procedure employed by Maguire and Haard (1976), the fluorescent measurements for the compound extracted from banana were carried out directly on the original chloroform-methanol mixture. The extracts from banana showed an excitation maximum at 350 nm and an emission peak around 440 nm (Maguire and Haard, 1976) which is the expected spectrum for lipofuscin. In the present study, however, the original extract of mango showed the excitation maximum at 350 nm but there was no emission in the range of 400-480 nm.

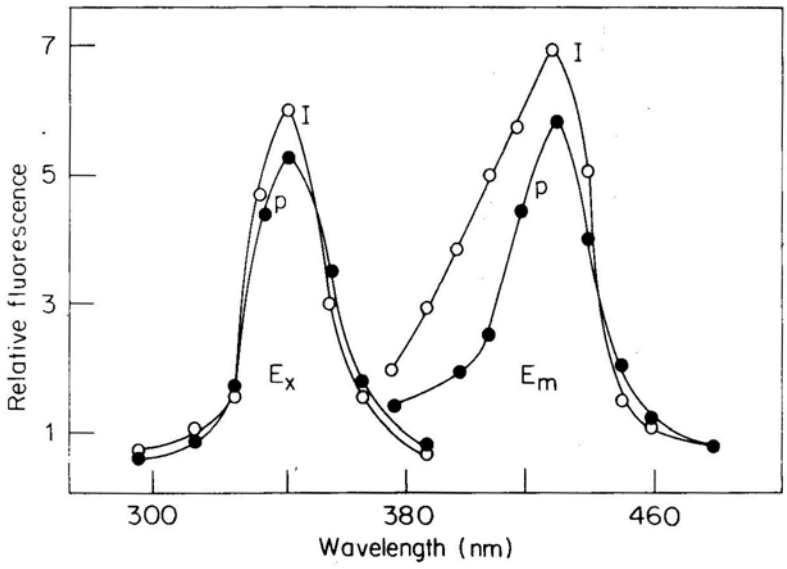

Figure 1. Excitation and emission spectra of fluorescent lipofuscin — like compound obtained after TLC separation.

To rule out the possibility of interference and quenching of fluorescence by the pigments present in the extract, as the original extract was highly coloured (0.3 ml of which contained 84 μg of total carotenoids in peel and 100 μg in pulp), it was necessary to remove the pigment from the extracts. After preliminary trials it was found that TLC on silica gel afforded a very good separation of the fluorescent compound from the pigments. Chromatography using petroleum ether followed by chloroform-methanol-petroleum ether was necessary for a clear separation. The fluorescent compound thus isolated showed excitation and emission maxima similar to that of lipofuscin *i. e.* 350 and 430 nm respectively (figure 1). Previous work has indicated the possibility of flavins and retinol-like compounds interfering with the estimation of the age pigment (Fletcher *et al.,* 1973). This interference is generally removed by washing the extract with water to remove phenolics and flavin like compounds, if any, and exposure of the extract to UV light to remove retinol and similar compounds (Fletcher *et al.,* 1973). Washing with water and UV exposure had no effect on the fluorescence spectrum of the compound isolated in the present study. Thus, it appears that in the case of coloured plant materials, removal of the colouring matter may be necessary to demonstrate the presence of the age pigment.

Recently (Knee, 1982) indicated that the procedure devised for lipofuscin estimation in animal tissues may not be applicable to plants because of the interference from phenolics and carotenoids. In the present study, by using TLC and using 2 different solvent systems, it has been possible to separate the lipofuscin-like compound from the interfering substances. The material thus separated was not a carotenoid or a phenolic compound but was a peptide. The fact that it is a lipid peroxidation product was confirmed by its positive reaction with thiobarbituric acid reagent. The hydrolysis of the fluorescent pigment fraction (Dowex-50 treated) yielded 7 amino acids separable by paper chromatography. However it is necessary to purify this lipoprotein to homogeneity for any further characterization and quantitization.

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