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Estimation of beta carotene from fruit peel wastes by high performance thin layer chromatography

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Abstract
Beta carotene is an important molecule that is precursor to vitamin A and possesses multiple health benefits. The present study plans to detect and quantify beta carotenes in fruit peel wastes collected from a beverage production center in Kolkata. Beta carotene was extracted by ethanol: hexane (4:3, *v/v*) solvent and the extracts were subjected for HPTLC analysis. The result showed presence of significant (P<0.05) amount of beta carotene in orange peels followed by mango peel and pomegranate peel. It could be concluded that feeding of orange peel wastes to animals might be beneficial due to presence of good amount of beta carotene in it.

Keywords: Beta carotene, HPTLC, mango peel, orange peel, pomegranate peel

Introduction
Researches confirmed good antioxidant activity in fruit peels. Large number of plant metabolites like phenolic acids, flavonoids, water soluble vitamins and beta carotenes, are responsible for this antioxidant activity [1, 2]. Beta carotenes are important member of antioxidant molecules that is available in fruits and vegetables. This molecule contains eight isoprene units joined to form a long conjugated chain. Beta carotene is the precursor of vitamin A (Retinol) and can ameliorate oxidative stress induced by reactive oxygen species in living animal systems. Beta carotene has not only nutritional value but also from economic point of view it is an important molecule [3].
Some researches [4] were carried out to detect and quantify beta carotene from vegetable wastes (mostly peels and pods), however very little work was done to quantify beta carotene from different fruit peel wastes. Fruit peel wastes, with antioxidant properties, are considered valuable feed materials for animals [5]. Objective of the present study is to identify and estimate beta carotene from three fruit peels collected from beverage industry as left over material. To the best of our knowledge this is the first reporting of beta carotene content by HPTLC method from peel waste samples collected from fruit beverage industry.

Materials and Methods
Sample Collection
Peel wastes of orange, mango and pomegranate were collected from a juice manufacturing centre located at Narendrapur situated in Kolkata, India. These waste materials were collected in sterilized plastic bins during the month of February – May'2018. Average moisture contents (Table 1) of the peel wastes were recorded during collection with a moisture meter (HE53, Metler Toledo, USA) at the day of collection and after shade drying.

Table 1: Cultivars and moisture contents of peel wastes

Sl. No	Fruit	Cultivar	Residue Collected	Moisture at collection (%)	Moisture after shade drying (%)	Abbreviated As
1	Orange (*Citrus reticulata*)	*Nagpur Mandarin*	Peel	75.57	11.55	OP
2	Mango (*Mangifera indica*)	*Totapuri*	Peel	65.69	13.43	MP
3	Pomegranate (*Punicagranatum*)	*Ruby*	Mixture of Peel, albedo and membrane	59.96	11.79	PP

Extraction of Sample
After collection, peels were washed with distilled water and then air-dried under shed for five days.

The peels were chopped into small pieces with a sharp scissor and then made it into coarse powder using an electrical grinder.

The coarse powder from each fruit peel was extracted as per the standard method (AOAC, 1984) [6] of beta carotene extraction with little modification. Briefly, 5g of sample extracted 5 minute with 35 ml ethanol: hexane (4:3, *v/v*) solvent in an orbital shaker. Re-extraction of the sample was done with the same volume of the extracting solvent and the extracts were pooled and stored in dark.

HPTLC Assay

Beta-carotene of the samples was estimated by the method described by Das *et al*. (2017) [7]. High performance thin layer chromatographic (HPTLC) analysis was carried out on a HPTLC plate pre-coated with silica gel. Samples (10µl) and standards (10-25 µl) were applied on plates by Linomat 5 applicator (Camag, Switzerland). The plates were developed to a distance of 90mm in Camag twin- trough chamber with mobile phase of Petroleum ether: Acetone (70:30, *v/v*)in 27 °C for 25 min. Afterwards, the plates were scanned for densitometry analysis in CAMAG TLC scanner (Camag, Switzerland) at λmax =450 nm. The chromatograms were finally integrated using Win CATS 4.0 computer programme.

Statistical Analysis

All the measurements were done in triplicates and analyzed by one way ANOVA using SPSS software version 16 (SPSS Inc., IBM). The mean values were compared by Duncan Multiple Range Test [8] at 5% significance level.

Result and Discussions

Densitometry analyses (Fig 1) in the present HPTLC assay identified beta carotene peaks in extractions of shade dried fruit peel wastes and standards. The band of beta carotene in the sample was confirmed by comparing Rf values (0.87) and spectra (Fig 2). Quantification of beta carotene concentration was done by five point standard curve (Y= -4217.948+4507.712X; r^2= 0.894).

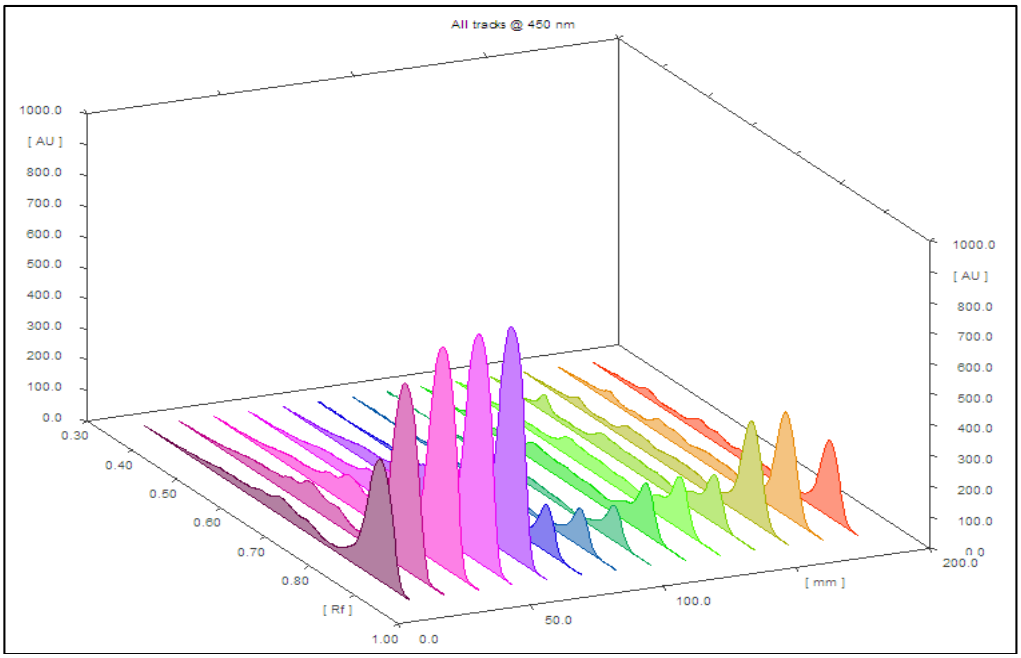

Fig 1: HPTLC Densitogram at 450nm in the order of standard beta carotene (tracks 1-5), PP(tracks 6-8), MP (tarcks 9-11) and OP (tracks 12-14)

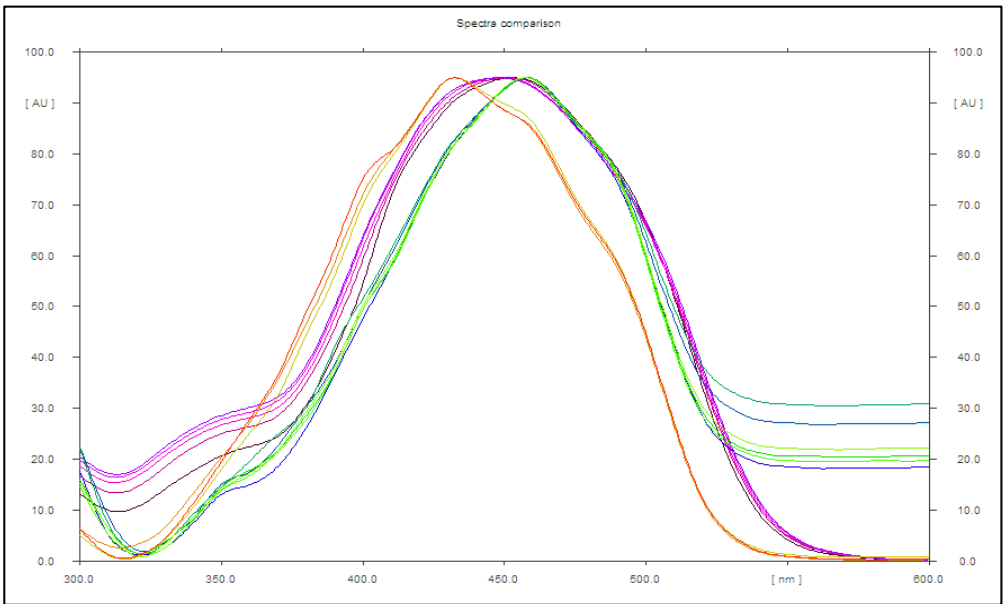

Fig 2: Spectra of standard beta carotene and other samples

Present study detected beta carotene in all three samples (Fig 1). Table 2 showed significantly (P<0.05) high concentration (4.99±0.04 mg/100g) of beta carotene in OP followed by MP (3.162±0.05 mg/100g) and PP (2.47±0.01mg/100g). John O. Onuh *et al*. [9] earlier reported beta carotene content of mango peels of various cultivars and the present study is in line with that. Rubashvili *et al*. [10] described carotenoid content of various agro-industrial wastes and stated that beta carotene content of orange peels ranging between 41.66 – 59.16 µg per gram of dried peel. Beta carotene content of pomegranate peel wastes could not be compared due to dearth of available literatures.

Table 2: Concentration (mg/100g) of beta carotene in peel wastes

	MP	OP	PP
Beta carotene	3.162± 0.05^{b}	4.99±0.04^{a}	2.47±0.01^{b}

Values are means ± SEM, n = 3 per treatment group. Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the DUNCAN test.

Although orange peel contains good amount of beta carotene, it is evident that fruit peels contain less beta carotene than vegetable wastes [4]. Antioxidant activity of fruit peels were extensively studied [9, 11, 12] worldwide. Present study clearly showed that presence of beta carotene in fruit peel wastes might be a contributing factor for its antioxidant activities.

Conclusion

Orange peels are widely used as natural colorant and flavoring agent in food processing industry. Present study clearly demonstrated that orange peel wastes could also be used sustainably in animal feed as it contains good amount of beta carotene that could be helpful for optimizing animal production. However further study should be carried out to identify various polyphenol compounds with bioactive roles in fruit peel wastes for their possible application in animal feed industry.

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