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Analysis of Physico-Chemical Properties of Natural Food Colours from Fruits and Vegetables

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The present study was conducted in the Department of Post Graduate And Research Center and Quality control lab, Rajendranagar, Hyderabad to synthesize the nano particles from selected fruits (papaya and black grapes) and vegetables (tomato & beet root) and physico chemical characteristics of extracted nano food colours (NFCs) was assessed. Selected fruits and vegetables are subjected to infrared drying (IR) and are grounded into fine powder. The amount of powder obtained after drying the samples of papaya, black grapes, tomato and beet root was 8.0gm, 11.5gm, 6.0gm and 12.0gm respectively for 100gm of fresh sample. Nano particles or NFCs were synthesized by oxalate decomposition method. NFCs thus obtained are subjected to scan under scanning electron microscope (SEM). The size of the NFCs ranged from 695.0-723.0 nm, 668.8-796.0 nm, 772.0-965.0 nm, and 859.0-991.0 nm in papaya, black grapes, tomato and beet root respectively. From the images scanned with SEM it was observed that the NFCs are spherical in shape coated with zinc oxide. The NFCs thus obtained were assessed for physico chemical characteristics (Yield, Colour quality, Concentration, Zinc and Bio active compounds).

Introduction

Color becomes the most sensitive part of any commodity not only for its appeal but also it enhances consumer acceptability. The demand for natural colours is increasing day by day because of awareness of positive health benefit out of natural compounds (Chattopadhyay *et al.*, 2008). The organic colour stuffs are obtained from roots, stems, leaves, barks, flowers and berries of various plants and from certain insects and shellfish. The inorganic pigments are insoluble salts which are precipitated (Paul *et al.*, 1996). There is a worldwide trend towards the use of natural additives and food colourant in food applications (Ghorpade *et al.*, 1995). Nanotechnology describes materials, devices and systems with structures and components exhibiting new and significantly improved physical, chemical and biological properties as well as the phenomena and processes enabled by the ability to control properties at nanoscale (Miyazaki and Islam, 2007).

A range of nano techniques and materials are being developed to control over food character traits (Gardener, 2002). Inorganic nanomaterials are stable under harsh process conditions but also generally regarded as safe to human beings and animals (Stoimenov *et al.*, 2002; Fu *et al.*, 2005). Potential applications of nanotechnology are formulation of food products, food packaging applications and new materials for food equipments, new sanitizers and also water purification (Enculescu *et al.,* 2008).

Materials and Methods

Raw materials

The fruits (papaya and black grapes) and vegetables (tomato and beet root) were procured from local market. Chemicals used in this experimentation and analysis were of food grade, purchased from standard Indian companies.

Preparation

Fruits (papaya and black grapes) and vegetables (tomato and beetroot) of each 1kg were thoroughly washed in hot water and were cut into thin pieces. These pieces are placed in separate trays and were subjected to Infrared (IR) drying. After drying, the samples were cooled and grinded in a conventional grinder into fine powder.

Synthesis of color nano particles

In the present study NFCs were synthesized using dry powders from selected fruits (papaya and black grapes) and vegetables (tomato and beet root) by oxalate decomposition method. Zinc oxide solution was prepared by mixing of equimolar concentration of zinc source and oxalic acid. Dried fruit and vegetables samples were ground into powder and analyze to higher temperature like 600°C. Powders were sieved using 2 micrometer sieve then 5% (v/v) extracts were prepared from respective powders. While stirring of 0.2% nano scale zinc oxide solution, 1% gum solution was added. Prepared 5% extract was added slowly with string and heating. Final solution was made up to 100 ml and solution was kept at room temperature for one hour for stabilization.

Physico-chemical analysis

NFCs extracted were subjected to analysis soon after their extraction for parameters like yield, colour quality, concentration, zinc, total carotene, anthocyanin, lycopene and total betalain content. Yield of colour was estimated using Weighment method on dry weight basis. Colour quality of the samples was estimated by using Hunterlab calorimeter (Colour Quest XE hunter Lab, USA). The procedure for estimation of zinc was given by Garcia *et al.,* (1974). The concentration of the colour was estimated by spectrophotometer as given by Jayaraman (1996). Procedures for estimation of total carotene, anthocyanin, lycopene and total betalain content were given by Ranganna (1986).

Physical quality attributes

Determination of particle size

Scanning Electron Microscope (SEM) Protocol

Samples were exposed against 2.5% gluteraldehyde in 0.1M phosphate buffer (pH 7.2) for 1 hr at room temperature. Dehydrated in silica dessicator for 1 hr. The processed samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of gold coat over the samples were done by using an automated sputter coater (Model-JEOL JFC-1600) for 3 minutes and scanned under Scanning Electron Microscope (SEM - Model: JOEL-JSM 5600) at required magnifications (John and Lonnie, 1998).

FT-IR measurements

The FT-IR measurements of the natural colour encapsulated nanoparticles were carried out with TENSOR-27 (Bruker). To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 15000 rpm for 15 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed.

Statistical analysis

The data were analyzed for difference of significance by ANOVA used CRD and CD values are presented.

Results and Discussion

Drying of samples and preparation of fine powder

The yield of powder obtained after drying and grinding of samples is given in table 1.

Particle size and structure

SEM

The NFCs synthesized from selected fruits (papaya and black grapes) and vegetables (tomato and beet root) were subjected to scan under Scanning Electron Microscope (SEM). From the images scanned with scanning electron microscope, it was found that the nano particles are spherical and agglomeralated in nature.

They are found intact with the coating material (Zno). The coating material is highly thick holding the nano particles inside. It was also observed that the size of the colour particles were in nano meters whereas the size of the coating material was in micro meters which showed that the zinc oxide was highly coated around the nano particles. The size of the NFCs obtained is given in table 2.

The results in the above table indicated that the size of the NFCs varied from sample to sample. The size of the NFCs synthesized from papaya ranged from 695.0-723.0 nm, from black grapes (668.0-796.0 nm), from tomato (772.0-965.0 nm) and from beet root it ranged from 859.0-991.0 nm. The average size of the particles were 713.0, 736.0, 859.0 and 925.0 nm in papaya, black grapes, tomato and beet root respectively.

FT-IR measurements

FT-IR results revealed that absorption bands were present at 3349, 1635 cm^{-1} in NFCs of grape, tomato and papaya. The absorption band at 3349 cm^{-1} is assigned to the N–H group or primary amides which are present in the respective colour extract. This also indicates the presence of alcohols with free OH.

The absorption band 1635cm^{-1} corresponds to the presence of nitrites and tertiary amides. Whereas, the absorption band 2102 cm^{-1} which is present in the sample Beet root NFC is assigned to the aromatic isonitriles which is absent in the other samples tested.

The results indicated that the colour of the respective extract was clearly encapsulated by the zinc nano particles. According to Cross, 1960 for trans fatty acid absorption bands were present at 1000-950 cm^{-1} and for cis fatty acid at 780- 680 cm^{-1} (Figs. 1–4).

DLS and zeta potential measurements

The particle size (DLS) and zeta potential measurements of the prepared samples were carried out using Nanopartica, SZ-100 (HORIBA). The hydrodynamic radius of the colour encapsulated nanoparticles was recorded as 123nm, 232nm, 385nm, 1005nm corresponds to Papaya, Tomato, Grapes and Beet root respectively.

The sizes (hydrodynamic radius) of NFCs synthesized are in good correlation with the sizes measured under SEM.

The higher zeta potential values (Grape: -40.5mV, Tomato: -36.5mV, Papaya: -32.7mV and Beatroot: -41mV) for all the samples tested indicate the high stability of the formed particles.

Yield

Yield of NFCs synthesized from selected fruits (papaya and black grapes) and vegetables (tomato and beet root) was calculated and recorded in the table 3.

Results illustrated in the table 3 indicated that the yield of nano particle solution was 160.0 ml from 8.0 gm of dry powder of papaya, 230.0 ml from 11.5 gm of black grapes, 120.0 ml from 6.0 gm of tomato and 240.0 ml from 12 gm of beet root for 100 gm of fresh sample.

Concentration

The results obtained for concentration of NFCs from selected fruits (papaya and black grapes) and vegetables (tomato and beet root) is given in the table 4.

In beet root, the concentration of colour was found to be higher (7.10), followed by black grapes (6.67), papaya (4.38) and least was for tomato (3.39). Between the vegetable samples the concentration of colour was found to be higher in beet root (7.10) and lower in tomato (3.39). In fruit samples the concentration of colour was found to be higher in black grapes (6.67) followed by papaya (4.38). The values obtained in the present study for concentration are much higher compared to the value reported by Priya Mandhana *et al.*, (2007). This may be mainly due to the synthesis of the colour nano particles with very small size and large surface area to volume ratio.

Table.1 Yield of powder obtained from the given samples after drying and grinding

Sample Sample	Papaya	Black grapes	Tomato	Beet root
Weight of powder in (gm)/1kg of fresh sample	80.0 (8.0)	115.0 (11.5)	60.0 (6.0)	120.0 (12.0)

***** Figures in parenthesis indicate percentages.

Table.2 Size of NFCs obtained from the selected fruits (papaya and black grapes) and vegetables (tomato and beet root)

Sample	**Range(nm)**	**Average (nm)**
Papaya	695.0-723.0	713.0
Black grapes	668.0-796.0	736.0
Tomato	772.0-965.0	859.0
Beet root	859.0-991.0	925.0

Table.3 Yield of NFCs synthesized from selected fruits (papaya and black grapes) and vegetables (tomato and beet root)

Sample	**Weight of the fresh sample (gm)**	**Weight of the dry powder (gm)**	**Volume of nano particle solution (ml)**
Papaya	100.0	8.0	160.0
Black grapes	100.0	11.5	230.0
Tomato	100.0	6.0	120.0
Beet root	100.0	12.0	240.0

Table.4 Concentration of NFCs from selected fruits (papaya and black grapes) and vegetables (tomato and beet root)

Sample	Papaya	Black grapes	Tomato	Beet root
Concentration	4.38	6.67	3.39	7.10

Table.5 Colour quality of NFCs synthesized from selected fruits (papaya and black grapes) and vegetables (tomato and beet root)

Sample	**Light (L)**	**Hue (a)**	**Brightness (b)**
Papaya	14.55	3.33	4.59
Black grapes	16.56	3.59	8.53
Tomato	14.26	2.73	5.45
Beet root	13.36	4.56	5.34

Table.6 Zinc content in the NFCs synthesized from selected fruits (papaya and black grapes) and vegetables (tomato and beet root)

Sample	Papaya	Black grapes	Tomato	Beet root
Zinc content (mg/100gm)	3.94	6.81	2.95	7.51

Table.7 Quantity of bio active compounds in NFCs synthesized from selected fruits (papaya and black grapes) and vegetables (tomato and beet root)

Bioactive compounds	**O.D value**	**Content in (100gm)**
Total carotenes (papaya)	0.31	92.16 µg
Anthocyanins (black grapes)	0.27	118.91 mg
Lycopene (tomato)	0.125	4.68 mg
Betalains (beet root)	2.2	5.28 mg

Fig.1 FT-IR spectrum of Papaya's NFC

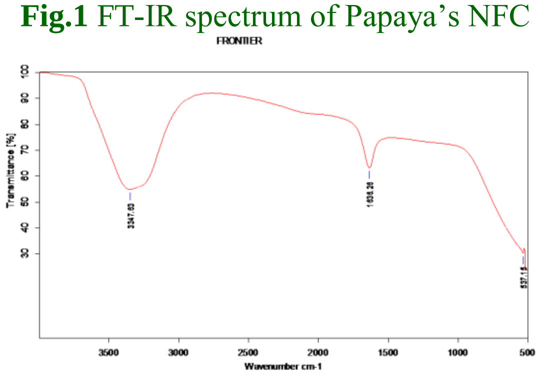


Fig.2 FT-IR spectrum of Black grapes's NFC

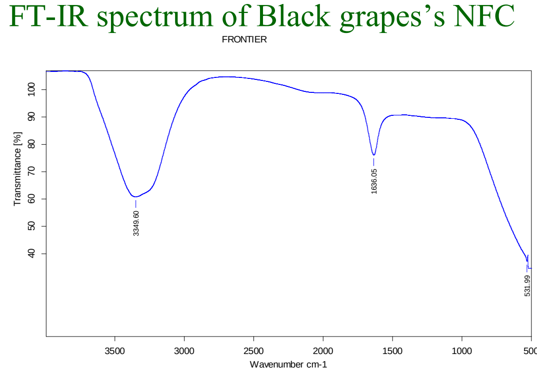
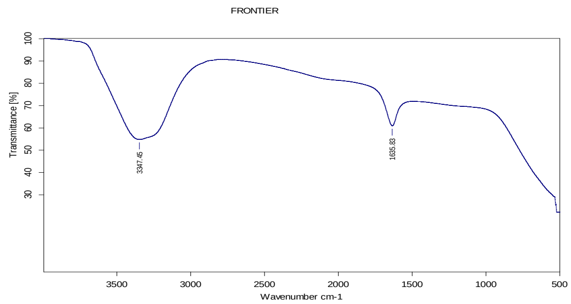
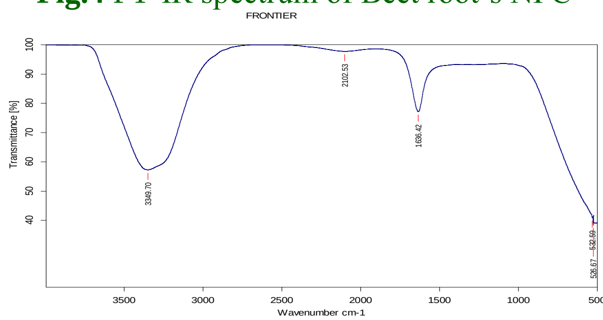


Fig.3 FT-IR spectrum of Tomato's NFC



`Fig.4 FT-IR spectrum of Beet root's NFC



Colour quality

The colour quality of NFCs synthesized from selected fruits (papaya and black grapes) and vegetables (tomato and beet root) is given in table 5.

The colour value was mainly determined in the form of light, hue and brightness. Among the vegetable samples lightness was found to be higher for NFC with tomato (14.26%) followed by NFC with beet root (13.36%). In the fruit samples lightness was found to be higher in NFC with black grapes (16.56%) and lower in NFC with papaya (14.55%). The values for lightness for all the NFCs were extended nearly towards '0' value i.e., black indicating good colour quality.

The + a (Hue) value which indicates more of redness and less of greenness was higher for beet root NFC (4.56%) compared to NFC with tomato (2.73%) among vegetable samples. In fruit samples hue value was higher for NFC with black grapes (3.59%) compared to NFC with papaya (3.33%). Between the vegetable samples the brightness was found to be higher for NFC with tomato (5.45%) and lower for NFC with beet root (5.34%) and in fruit samples higher for NFC with black grapes (8.53%) compared to NFC with papaya (4.59%).

Zinc

The nano particles synthesized from selected fruits (papaya and black grapes) and vegetables (tomato and beet root) in the present study are coated with zinc oxide. Hence zinc was estimated in the NFCs to know the amount of zinc that is present in the given nano particle solution. As zinc rich foods are tend to be expensive, the daily intakes of zinc was much lesser to the requirement. The recommended daily intake of zinc is 15mg/day. The amount of zinc in the given NFCs of selected fruits (papaya and black grapes) and vegetables (tomato and beet root) is given in the table 6.

Results illustrated in the table 6 indicated that the zinc content in NFCs synthesized from papaya was 3.94 mg/100gm, 6.81mg in black grapes, 2.95mg in tomato and 7.51 mg/100gm in beet root. In fresh samples of tomato and beet root the zinc content was 0.41 and 0.91 respectively where as in papaya and black grapes it was not reported (Gopalan *et al.*, 2007). When compared to the above values, it was observed that, the Zinc content of NFCs synthesized was higher indicating the significance of NFCs in enhancing the Zinc content of the food products. Zinc and zinc oxide were used as nutritional additives and antimicrobial agents in food packaging (Brunner, 2006). Hence these NFCs also can be used as nutritional additives and also as antimicrobial agents.

Bioactive compounds

The quantity of bio active compounds were estimated in the NFCs synthesized from selected fruits such as total carotenes (papaya) and anthocyanins (black grapes) and vegetables such as lycopene (tomato) and betalains (beet root) and results are given in table 7.

Results in the table 7 indicated that the total carotene content in NFC with papaya was 92.16 µg/100gms. The anthocyanin content in NFC with black grapes was 118.91 mg/100gm. The lycopene content in NFC with tomato was 4.68 mg and betalain content in NFC with beet root was 5.28 mg/100gm.

Thus it can be concluded that it is feasible to synthesize NFCs from dehydrated powders of selected fruits (papaya and black grapes) and vegetables (tomato and beet root) with good physico chemical (yield, colour quality, concentration, zinc and bio active compounds) properties.

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