Full Length

DEVELOPMENT OF FOOD SPOILAGE INDICATOR FOR DAIRY PRODUCTS USING ANTHOCYANIN EXTRACTED FROM *HYLOCEREUS POLYRHIZUS*

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ABSTRACT

Intelligent spoilage indicators are the emerging area of food packaging sector as it can provide information on quality of food during storage and transportation. The present research work was aimed to develop an anthocyanin based food spoilage indicator to detect spoilage in dairy products. Anthocyanin was extracted from the peels of dragon fruit, incorporated into filter paper and used as food spoilage sensing agent. Dragon fruit peel extract showed a characteristic peak of absorption at 537nm and was found to have 141.21±3.9 mg/g of total anthocyanin content and 46.8 ± 0.98 mg/g GAE of phenolic compounds. The pH sensitivity of the dragon fruit peel extract and indicator film were quite similar with the colour change from red to yellow as the pH increases. The indicator film was effective insignalling the quality deteriorations occurring in stored paneer and khoa.

Key words: anthocyanin, food spoilage indicator, dragon fruit peel, paneer, khoa

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INTRODUCTION

Considerable portion of overall income of Indian farmers is mainly generated from the volume of milk produced from cattle rearing (Bhardwaj *et al.,* 2023). India ranks first in world milk production, contributing 25% of global milk production. Milk processing capacity of Indian dairy industries was calculated as 126 million litres / day in 2023. The Indian milk production has registered 58% raise in past nine years (2014-23) with 6% CAGR and reached 230.58 million tons in the year 2022-23 (Invest India, 2024).

As dairy products are highly perishable in nature, rapid market growth in India requires significant support in shelf life extension of milk and value-added dairy products through infrastructural development and implementation of

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innovative technologies (Barukčić *et al.,* 2021).

Paneer is an Indian soft cheese variety with minimal shelf life of a day at ambient temperature and about 5-6 days at refrigeration temperature. Although the thermal milk treatments destroyed all the spoilage and pathogenic micro-organisms, further handling and washing of coagulated curd can reintroduce spoilage organisms (Prajapati *et al.,* 2023). Khoa is a versatile traditional dairy product with high water activity and easily available nutrients which renders easier microbial proliferation and enzymatic spoilage. The shelf life of khoa is about 3–5 days at ambient temperature and 7–10 days at refrigerated temperature (Badola *et al.,* 2023).

Application of biosensors in food packaging to develop an intelligent packaging system have gained attention as they can emit a signal (electric, colorimetric, or others) in real time in response to any changes in the initial packaging conditions and food quality. In the beginning of the deterioration process, food undergoes a change in pH which will be measured by the pH indicator and could indicate the quality of the food at the time of purchase or before consumption, making it safer for consumers (Balbinot-Alfaro *et al.,* 2019).

pH-based colorimetric dyes can be extracted from various natural or synthetic sources. The higher toxicity of pH sensitive chemosynthetic dyes such as xylenol blue, methyl red, bromocresol green, and bromophenol blue limits their usage in developing food spoilage sensing packaging films. Natural plantbased pigments such as anthocyanin, curcumin, betalain are also capable of indicating the food spoilage as they are pH sensitive. These dyes are nontoxic, ecofriendly and safe to both human body and environment(Azlim *et al.,* 2022; Balbinot-Alfaro *et al.,* 2019).

Anthocyanins are water soluble phenolic compounds responsible for the red, purple and blue hues of plant leaves, flowers and fruits. Anthocyanin holds enormous health benefits with its antioxidant, anti-inflammatory, antidiabetic and anti-cancerous properties (Nisha *et al.,* 2022). Red cabbage, blueberry, eggplants, moringa leaves, dragon fruit have considerably high amount of anthocyanin (Vo *et al.,* 2019; Nisha *et al.,* 2022; Azlim *et al.,* 2022).

The peel of red dragon fruit (*Hylocereus polyrhizus*) contains natural, red coloured, cyanidin-3-glucoside type anthocyanin pigments (Herlina *et al.,* 2021). It is rich in nutraceutical active compounds like antioxidants, vitamin C, minerals, fibre and phytoalbumins. The peel of dragon fruit has higher antioxidants and phenolic content than the pulp and thus could be a good source of active compounds like chlorogenic acid, gallic acid, and quercetin (Chia and Chong, 2015).

Current research is focused on recovering the peels of dragon fruits and turning them into a pH sensitive food spoilage indicator as a means of achieving sustainable development (Rawdkuen and Kaewprachu, 2019).

MATERIALS AND METHODS

Materials

Peels of dragon fruit (*Hylocereus polyrhizus*) were obtained from the local shops of Chennai, Tamil Nadu, India. Other analytical chemical reagents, such as ethanol, methanol, acetic acid, chloroform, 2, 2-diphenyl-1-picryl hydrazyl-hydrate (DPPH), gallic acid, Folin-Ciocalteureagent, sodium carbonate and Whatman filter paper 2 were purchased from Sisco Research Laboratories Pvt. Ltd., India.

Extraction of anthocyanin from dragon fruit peel

Extraction of anthocyanin from dragon fruit peels was carried out as per the method suggested by Taghavi *et al.* (2022) with methanol and chloroform as extraction solvents. A buffer solution was prepared by mixing 30 mL of methanol, 20 mL of distilled water and 0.3 mL of acetic acid. The solvent mixture was prepared by mixing 1:1 ratio of buffer solution with chloroform. The sample to solvent ratio was maintained at 1:20, for which 1g of sample was mixed with 20 mL of solvent mixture. Then the setup was incubated at 4°C for 48 hrs in the dark and centrifuged at 4°C, 7000 rpm for 15 minutes in a refrigerated centrifuge (Sorvall ST1R Plus-MD, Thermo Fisher, Germany). The supernatant was then removed and stored at 4°C for further analysis.

Spectral characterisation of the dragon fruit peel extract

The dragon fruit peel extract (DFPE) was read using UV-Visible absorption spectra at 200-800 nm with microplate reader (Epoch microplate spectrophotometer, BioTek Instruments, Inc., United States) and 96 well plates (Taghavi *et al.,* 2022).

Total anthocyanin content

The total anthocyanin content of DFPE was estimated based on the method described by Khoo *et al.* (2022). The absorbance of 200 µL DFPE was measured at 537 nm, as the maximum absorption of the extract solution was 537 nm. Total anthocyanin content was calculated based on the equation as follows:

$$\textit{Total anthocyanin content (mg/g)} = \frac{(A \times V \times D)}{(98.2 \times L \times m)}$$

Where,

A is the absorption at 537 nm;

V is the volume of the extract solution (mL);

D is the dilution factor;

98.2 is extinction coefficient (M-1cm-1);

L is the path length and

m is the mass of DFP.

Total phenolic content of dragon fruit peel extract

Total phenolic content of the dragon fruit peel extract was spectroscopically estimated by Folin-Ciocalteu method as suggested in Kupina *et al. (*2018). The absorbance was measured at the wave length of 765 nm with micro plate reader. The results were expressed as mg/g gallic acid equivalent (GAE) by using the following equation,

$$\textit{Total Phenol (mg/g)} = ((A-b)/m) \times ((V \times D)/(W \times 1000)) \times 100$$

Where,

A is absorbance of the sample test solution at 765 nm;

b is the y-intercept of the calibration curve;

m is the slope of the calibration curve;

V is the volume of the sample test solution;

D is the dilution factor;

W is the weight of the test material.

Antioxidant activity of the dragon fruit peel extract

DPPH was used to evaluate the antioxidant activity of dragon fruit peel extract. In brief, 100 µL aliquot of different concentrations of anthocyanin extract was added to 100 µL of 0.1 mM DPPH in ethanol contained in flat bottom 96 well plates and incubated at room temperature for 30 min in darkness. Ethanolic DPPH was used as blank control, while aqueous ascorbic acid (1 mg/mL) was used as a reference to compare the antioxidant activity of the anthocyanin extract. After incubation, absorbance was read at 517 nm using a microplate reader. The percentage radical scavenging activity or inhibition percentage was calculated using the following equation, (Mushtaq *et al.,* 2024)

$$\% \text{ Radical scavenging activity} = (A_c - A_s) / A_c \times 100$$

Where,

Ac - absorbance of the control;

As - absorbance of the sample or standard sample.

pH sensitivityof dragon fruit peel extract

1 mL of dragon fruit peel extract was mixed with series of buffer solutions of pH varying from 3-10, so that a final volume of 2 mL was obtained. The solution was then incubated for 30 min and the colour change was observed (Santoso *et al.,* 2023).

Phytochemical screening of dragon fruit peel extract

Phytochemical screening of dragon fruit peel extract was carried out with gas chromatography mass spectrometry (GCMS) (Shimadzu, QP2010 PLUS). The fresh dragon fruit peel extract was filtered using 0.3 micron syringe filter for GCMS analysis (Vergheese and Vishal, 2018).

Preparation of spoilage indicator paper

Whatman filter paper was cut into a circle of 9 cm diameter and placed in a petri plate. 25 mL of DFPE was poured into the petri plate and left aside over night to let the anthocyanin to get absorbed into the filter paper and dip coated with 3% poly lactic acid polymer dissolved in chloroform to immobilize the incorporated anthocyanin. This anthocyanin incorporated paper was used as the spoilage indicator.

Colour analysis of spoilage indicator paper

Hunter Lab Mini scan XE plus colorimeter (Color Ques XE) was used to measure the colour of developed spoilage indicator paper. The final results were presented as L (lightness: 0 = black, 100 = white), a (−a = greenness, +a = redness), b (−b = blueness, +b = yellowness), H

(Hue angle: 0° = red, 90° = yellow, 180 = green, 270 = blue) values, each as mean and standard error of triplicate samples. Chroma indicates the purity of colour with values ranging from 0 to 131 where the lower chroma indicates less intense colour and higher chroma indicates the high intense colour (Chia and Chong, 2015; Konika Minolta, Inc., no date). The hue angle and chroma were calculated with the following equations,

$$\text{Hue angle}(H) = \text{Tan}^{-1}(b/a)$$

$$\textit{Chroma}\ (C) = \sqrt{(a^2+b^2)}$$

pH sensitivity of spoilage indicator paper

The pH sensitivity of developed spoilage indicator paper was assessed as per the method suggested by Nazri *et al.* (2022) with slight modification. The spoilage indicator paper was cut into 1x1cm pieces and immersed in 5 mL of buffer solutions of pH ranging from 3 to 12 for 5 min and the colour change was observed.

Dye leaching property of spoilage indicator paper

The dye leaching property of the developed pH indicator paper was evaluated by the method suggested by Ghorbani *et al.* (2021). The developed spoilage indicator paper was cut into 2 cm × 2 cm size and immersed in 10 mL of sterile distilled water. 200 µl of this distilled water was read at 537 nm at 0 minute, 30 minutes, 2 hrs, 4 hrs and 6 hrs by microplate reader.

Application of indicator paper in food packaging

The developed spoilage indicator paper was cut into1 cm x1cm pieces and placed inside polylactic acid pouches of 3 cm x 4 cm dimensions. 5g of paneer and 5g of khoa was packed in polylactic acid pouches and stored at 4°C for a period of 7 days and the colour changes occurring in the indicator film was observed.

RESULTS AND DISCUSSION

Spectral characterisation of the dragon fruit peel extract

The Fig.1 indicates the characteristic peak obtained at 537nm signifying the presence of anthocyanin in the dragon fruit peel extract. Similar spectral results were observed by Taghavi *et al.* (2022) with characteristic peak at 530 nm and by Khoo *et al.* (2022) with characteristic peak at 538 nm.

Total anthocyanin content and total phenolic content of dragon fruit peel extract

The total anthocyanin content of the dragon fruit peel extract was estimated as 21.29 ± 3.9 mg/g. Khoo *et al.* (2022) reported that 19.21 ± 0.01 mg/g of anthocyanin was present in dragon fruit peel and 54 ± 0.8 mg/100g GAE (gallic acid equivalent) of total phenolic content was present in the dragon fruit peel extract. Mello *et al.* (2014) also discussed that the dragon fruit peel contains 40.68 mg/g GAE of phenolic compounds.

GCMS analysis of dragon fruit peel extract

The results pertaining to GC-MS analysis of the methanolic extract of dragon fruit peels confirmed the presence of various active phenolic components in the dragon fruit peel extract. The compound prediction is based on WILEY 8.LIB and National Institute Standard and Technological Database (NIST 18) library. The results of the GC-MS analysis were tabulated in Table 1 and Fig.2 depicts the GC-MS spectra of DFPE.

Based on the area coverage of the compounds, ethyl L-menthyl carbonate occupied largest area 14.64 % at 20.364 minute, followed by oleic acid, 3-(octadecyloxy) propyl ester with 9.40 % at 24.147 minute and then 2- hydroxy- 2-ethyl- N2- (3-phenylpropenylideno) with 7.72 % at 24.375 minute. 14.64% area was covered by ethyl L-menthyl carbonate as methanol is used as extraction solvent. 9,12 - octadecadienoic acid occupied 4.53% area at 23.715 minute. Similar results were obtained by Vijayakumar *et al.* (2018) with 2.69 % area coverage for octadecadienoic acid and 6.17 % area coverage for oleic acid.

Antioxidant activity of dragon fruit peel extract

The results of DPPH radical scavenging assay conducted on anthocyanin extract against ascorbic acid (standard) was presented in Table 2. The dragon fruit peel extract showed 71.55 ± 0.57% antioxidant activity at 250 µg/mL concentration, while ascorbic acid showed 85.39 ± 0.38% antioxidant activity at the same concentration. At lower concentration of 7.81 µg/mL dragon fruit peel extract showed 4.71 ± 0.26 % antioxidant activity, while ascorbic acid showed 24.38 ± 0.46 % antioxidant activity at the same concentration.

The antioxidant activity is mainly due to the phenolic components present in dragon fruit peels, such as phenolic acids, flavonoids and phenolic diterpenes. These antioxidants can extend the stability of the developed indicator paper over time by inhibiting the initiation or propagation of oxidative chain reactions (Mushtaq *et al.,* 2024).

pH sensitivity of dragon fruit peel extract

The colour changes observed by exposing dragon fruit peel extract to different pH conditionswas illustrated in Fig.3. The phenomenon behind the observed colour changes was explained by Li *et al.* (2023). As anthocyanins are in the form of 2-phenylbenzopyranan cation, they remain red in colour at pH 1 to 4 and turn into carbinol pseudobase form from pH 4 to 6. At pH 8 to 10, they transform into purplish quinone and as the pH increases, it turns yellowish due to chalcone ring.

LAB colour analysis of indicator paper

The results of LAB colour analysis were tabulated in Table 3. The results of LAB colour analysis indicated the characteristic colour of the developed indicator paper. The “L” value was estimated as 36.61 ± 1.02, which indicates the brightness of the indicator paper. The “a” value was found

as 37.42 ± 0.93 and the "b" was 4.47 ± 1.17, which indicates the dark redness and slight yellowness of the film respectively (Chia and Chong, 2015). The hue angle was calculated as 0.56° ± 0.07, which indicates the redness of the developed indicator paper. The lower chroma value of 37.38 ± 0.78 indicates the lower intensity of red colour (Konika Minolta, Inc., no date).

pH sensitivity of indicator paper

The colour changes observed by exposing the developed indicator paper to different pH conditions was illustrated in Fig.4. The results were supported by the colour changes exhibited by dragon fruit peel extract.The colour of the indicator film changed from pink to pale yellow with pH 3-12, which was in accordance with the colour changes observed in dragon fruit peel extract.

Dye leaching property of indicator paper

Fig. 5 illustrates the dye leaching property of indicator paper during 24 hrs timespan. As the time increased, the intensity of the peaks increased. This gradually increasing peaks indicate the gradual leaching of anthocyanin from paper matrix to the distilled water. This might be due to the inability of the PLA coating to immobilize the anthocyanin within the matrix of the base material, which is the filter paper. In contrast, the intelligent indicator film developed by Ghorbani *et al.* (2021) with polylactic acid (PLA)/ polyethylene glycol (PEG)/calcium bentonite (CB) blend and *Malva sylvestris* anthocyanin extract showed low leaching rate of anthocyanins indicating a strong bond binding between the PLA composite and the anthocyanins.

Effectiveness of the developed indicator paper

The effectiveness of the developed spoilage indicator paper was studied on dairy products such as paneer and khoa which were stored at 4°C for a period of 7 days. The colour changes developed during the spoilage process was illustrated in Fig.6. The colour of the indicator paper was initially pink, indicating the acidic condition on the surface of paneer and khoa. The colour changed from pink to pale yellow, as the days progressed. This was because the dairy products were contaminated with micro organisms that could decompose proteins into polypeptides, amino acids and other compounds (Vo *et al.,* 2019). In brief, these results revealed the ability of the developed indicator film in timely sensing the spoilage with colour change.

CONCLUSION

This research was proposed to emphasise the importance of food safety, as safe food is essential for sustainable and healthy lifestyle in today's fast paced world. The developed food spoilage indicator utilizes the pH sensitivity of anthocyanin extracted from dragon fruit peels for timely indication of food spoilage. The pH sensitive efficacy of developed food spoilage indicator was studied *in vitro* and in the presence of dairy products such as paneer and khoa for food spoil age monitoring. Despite the effective and vast applications, higher dye leaching rate of the spoilage indicator paper

makes it difficult to industrialise it in larger scale. Further research and development are required to strongly immobilise the anthocyanin within the matrix of the filter paper.

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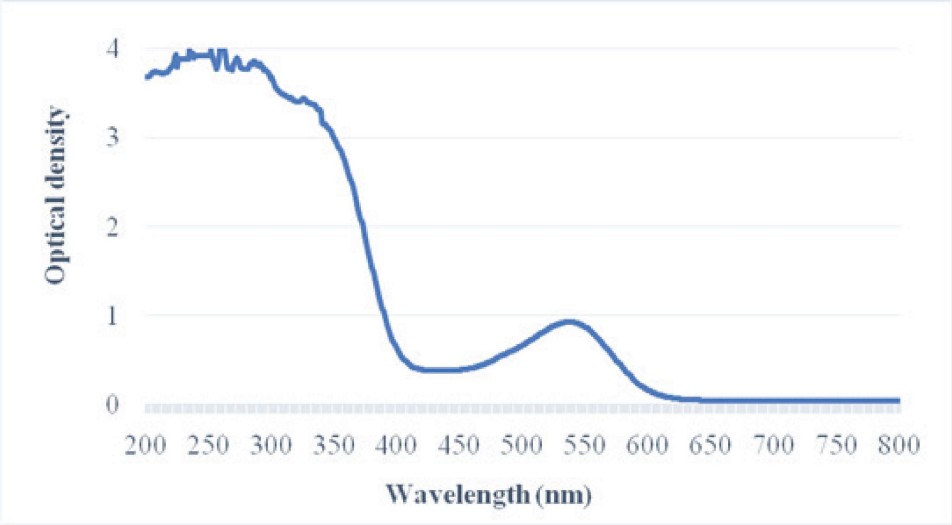

Fig.1. Characteristic peak of anthocyanin at 537 nm

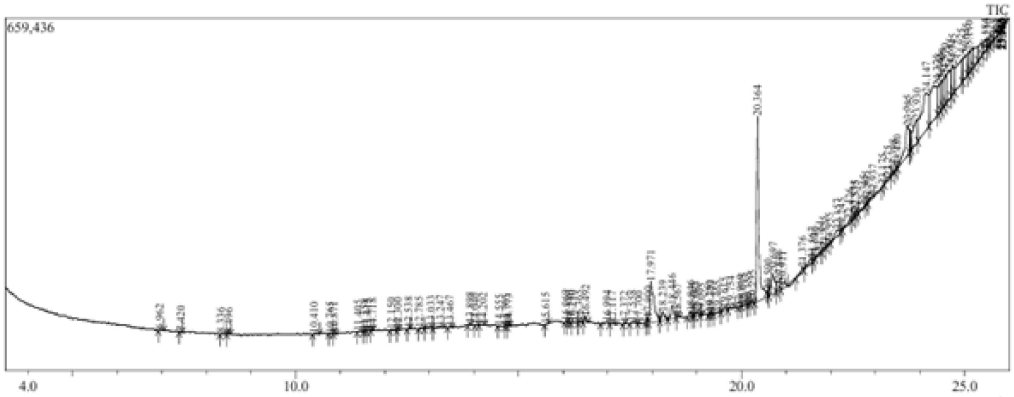

Fig.2. GCMS spectra of dragon fruit peel extract

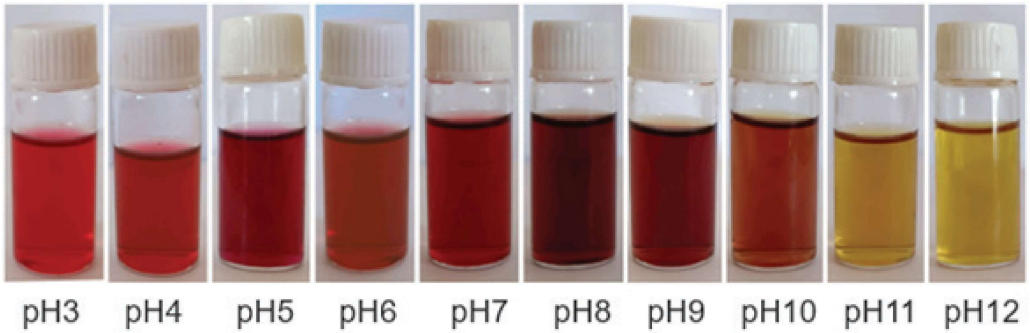

Fig.3. Change of colour observed in dragon fruit peel extract at different pH ranges

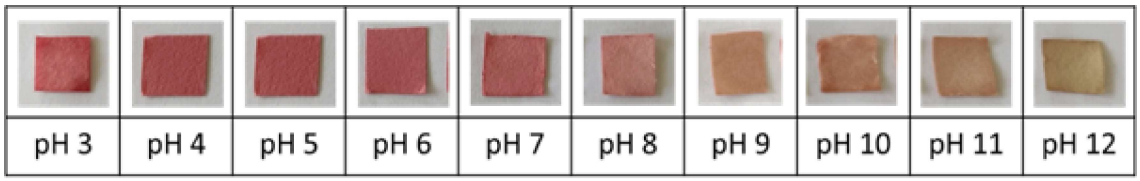

Fig.4. Change of colour observed in the developed indicator paper incorporated with dragon fruit peel extract at different pH ranges

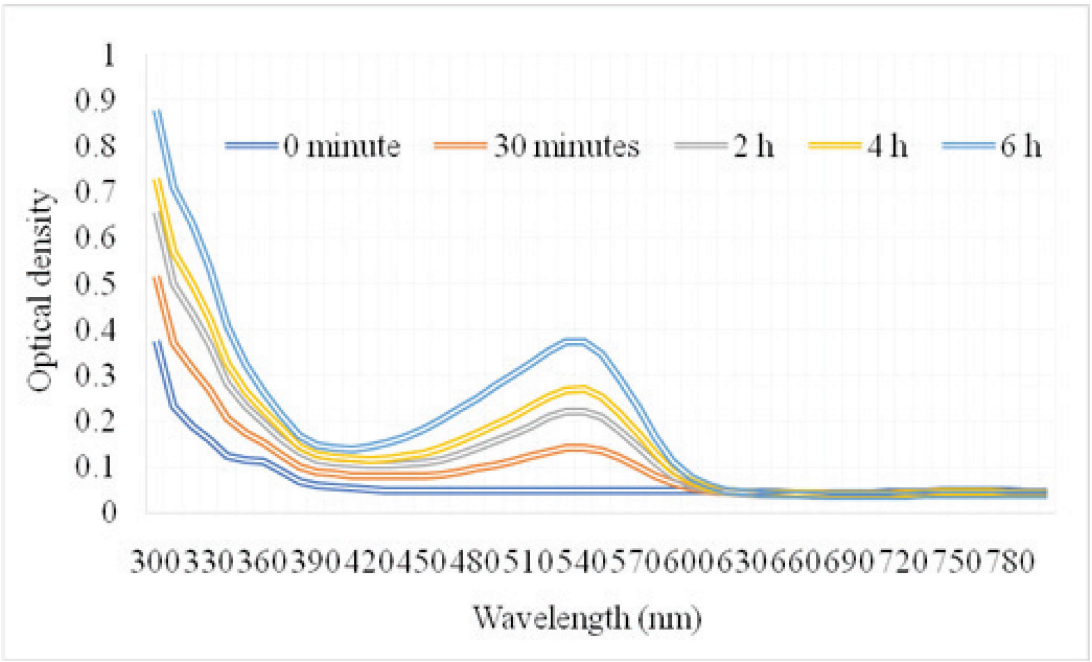

Fig.5. Dye leaching property of indicator paper

Food product	Day 1	Day 4	Day 7
Paneer			
Khoa			

Fig.6. Colour changes observed during the spoilage process

Table 1. GCMS analysis of dragon fruit peel extract

S. No.	Retention time (min)	Area (%)	Name of the compound
1	17.971	5.56	Neophytadiene
2	18.239	1.03	1-Pentadecyne
3	18.446	1.37	Oxirane, tetradecane
4	20.364	14.64	Ethyl L-menthyl carbonate- methanol
5	20.697	2.65	(Z)-Methyl heptadec-9-enoate
6	20.846	1.18	5-formyl-2-methoxyphenyl propionate
7	23.715	4.53	9,12-Octadecadienoic acid
8	23.775	1.18	Dimethylmalonic acid, ethyl pentadecyl ester
9	23.930	3.74	2-propenamide, n,n-bis(2-oxopropyl)-compound
10	24.147	9.40	Oleic acid, 3-(octadecyloxy)propyl ester
11	24.375	7.72	Butanehydrazide, 2-hydroxy-2-ethyl-N2-(3-phenylpropenylideno)
12	24.570	2.57	Quinoline, 4-chloro-6-methoxy-2-methyl compound
13	24.685	3.76	2-(2-Cyclohexyl-1-hydroxy-2-methoxyethyl)-3-methyl-1,4-dioxaspiro[5.4]decane
14	24.925	4.90	1-[3-Methyl-4-nitrophenyl]-3-amidinourea
15	25.045	3.33	cholest-5-en-3-yl (9z)-9-octadecenoate
16	25.110	1.82	Nepetalactone
17	25.184	1.76	1-Amino-2-(dimethylamino)-4-hydroxyanthracene-9,10-dione
18	25.230	1.96	benzenamine, 2,6-dichloro-4-nitro compound
19	25.368	1.63	diazene, bis[2-(3-methylpentyl)phenyl]-, 1- oxide
20	25.475	1.42	Dimethylsilyl 2, 3, 4, 6- o- dimethylsilyl-glucopyranose

Table 2. Antioxidant activity of DFPE at different concentrations

Concentration (µg/ml)	Radical scavenging activity of ascorbic acid (%)	Radical scavenging activity of dragon fruit peel extract (%)
250	85.39 ± 0.38	71.55 ± 0.57
125	84.98 ± 0.38	70.32 ± 0.29
62.5	84.51 ± 0.57	40.69 ± 0.20
31.25	83.45 ± 0.11	17.2 ± 0.18
15.63	51.83 ± 0.39	7.42 ± 0.26
7.81	24.38 ± 0.46	4.71 ± 0.26

Data are mean ± SE, n=6

Table 3. LAB colour analysis of indicator paper

S. no	Parameter	Results
1	L	36.61 ± 1.02
2	a	37.42 ± 0.93
3	b	4.47 ± 1.17
4	Hue	0.56° ± 0.07
5	Chroma	37.38 ± 0.78

Data are mean ± SE, n=3

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