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Full Length Research Article

pH Responsive Anthocyanin Filter Paper as a Visual Indicator for Volatile Gases and Monitoring Freshness of Meat

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Abstract

Recently, researchers are interested in fabricating smart packing material that shows colour change related to the food degradation for the real-time evaluation of quality and freshness of packed food. In this present study, anthocyanin-based extracts from various sources like red cabbage (RC), idli flower (IF), arali flower (AF) and vadamalli (VM) are prepared. The pH responsive colour changes of extracts was analyzed and UV-visible spectra were recorded for the four extracts in the pH range of 3-11. UV-visible spectra of extracts showed a band at approximately 530 nm in acidic pH which is shifted to 537 nm in neutral pH and 370 nm at basic pH. RC extract showed much clear colour change and appreciable UV-visible spectral change between the pH of investigation. Further, RC extract was coated on filter paper by dip and dry method. It was used for visual detection of volatile gases HCl and NH_3 that showed dark pink colour and green colour on exposing HCl and NH_3vapours, respectively. Besides, the extract coated filter paper was used for the analysis of goat meat degradation. The colour of the filter paper changed from pink to light green due to the degradation of meat products that releases NH_3.

***Key words*:** Anthocyanins, Red cabbage, UV-visible spectrum, Visual detection, Volatile gas sensing, Meat freshness

Anthocyanins are water soluble naturally available pigment molecule which are available in different colours like purple, red, blue, green depending on its source [Amogne et al. 2020; Farooq et al. 2020; Khazaei et al. 2016]. These are secondary metabolites of various fruits and flowers namely red cabbage, blueberry, red radish, black berries etc [Silva et al. 2017; Ekici et al. 2014]. The structure of anthocyanin contains 15 carbon atoms in its skeletal structure along with a few hydroxyl and methyl groups and an aromatic moiety [Herrman et al. 2020; Rodriguez-Amaya 2019]. Anthocyanins are stable over wide range of pH and show different colours due to the protonation and deprotonation of phenolic group of anthocyanins [Jing et al. 2008; Bueno et al. 2012]. Hence anthocyanin-based compounds are utilized in pH sensing applications [Pereira Jr et al. 2015; Liang et al. 2019; Yoshida et al. 2014; Zhai et al. 2017]. To monitor the freshness of milk and meat products, pH monitoring is an important tool [He et al. 2022; Kim et al. 2017]. pH of fresh milk ranges from 6.6-6.9. As the milk is spoiled, the pH of milk is changed to a pH ~4 due to the formation of lactic acid [Weston et al. 2020]. Similarly, the pH of meat products increases when it is spoiled or degraded [Hamoen et al. 2013]. The degradation of meat products results in the formation of NH_3 that changes the pH of meat products to basic pH.

As the colour of anthocyanins extracts are extremely sensitive to the pH, anthocyanin coated films can be used as an ideal candidate to monitor the freshness of milk as well as meat products.For instance, anthocyanins prepared from red cabbage extract was used as pH sensor as well as to prepare intelligent packing material [Pereira Jr et al. 2015]. Wang et al. reported the NH_3 vapour sensing using the intelligent film prepared using red cabbage anthocyanins and Artemisia sphaerocephala Krasch gum [Liang et al. 2019]. Tirtashi et al. used carrot anthocyanin incorporated on cellulose/chitosan composite films and used them as intelligent packing material for the monitoring the freshness of milk [Tirtashi et al. 2019]. Weston et al. also developed visual indicator to monitor the freshness of milk using red cabbage extract [Weston et al. 2020]. Alizadeh-Sani et al. developed barberry anthocyanin incorporated methylcellulose/chitosan nanofibers for monitoring the freshness of meat products [Alizadeh-Saniet al. 2021]. Zhou et al. reported the fabrication of double layer indicator films prepared using carrageenan, curcumin and/or anthocyanin and the emulsified layer made of konjac glucomannan. This double

film was used for the monitoring chicken meat products [Zhou et al. 2021]. In the present study, anthocyanin extracts are prepared from four different sources and their pH sensitive response are studied using UV-visible spectroscopy. Then, the extract was coated on filter paper using dip and dry method and used as alternative to pH paper to monitor the freshness of meat products and to sense volatile gases like NH_3 and HCl vapours.

MATERIALS AND METHODS

Ethanol, sodium hydroxide (NaOH), hydrochloric acid (HCl), phosphoric acid (H_3PO_4) and ammonium hydroxide (NH_4OH) were purchased from CDH. Monosodium hydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Merck and were used for the preparation of buffer solution. All reagents were used as received. Fresh red cabbage was purchased from local market. Vadamalli (*Gomphrena globosa*), Arali flower (*Nerium oleander*) and Idly flower (*Ixora coccinea*) were collected from the local gardens. Double distilled water was used throughout the experiments.

Instrumentation

UV-vis absorption spectra were recorded with a JASCO V-630spectrophotometer. pH meter with glass electrodes was used for measuring the pH of the solution.

Extraction of anthocyanin-based extracts

Anthocyanins were extracted from red cabbage (Brassica Oleracea), Vadamalli (Gomphrena globosa), Arali flower (Nerium oleander) and Idly flower (Ixora coccinea). Approximately 150 g of each anthocyanin chopped source was taken in a beaker and approximately 150 mL of distilled water was added, and the solution is heated to boiling for 20 min. Then, the solution was kept aside for 2 h and the mixture was filtered off using filter paper and extract was collected and stored in refrigerator. No further purification of extract was carried out and hence it is expected that diverse byproducts like sugars, amino acids, proteins etc., are present in the extract. The extracts are abbreviated as AF (arali flower extract), IF (idli flower extract), RC (red cabbage extract) and VM (vadamalli extract).

Preparation of anthocyanin extract coated filter paper

For coating anthocyanin coated filter paper, dry filter paper (2 × 3 cm) was taken and immersed in the extract solution for 5 min. Then, the filter paper was taken, dried using hair drier and kept in desiccator for 24 hours before using it.

Monitoring the freshness of mean products

For monitoring the freshness of meat, fresh meat products were collected from the local markets. It is washed with distilled water and kept in beaker. The freshly prepared anthocyanin extract coated filter paper was placed inside the beaker and the color of the film was monitored. Samples were then stored at room temperature (25°C).

Visual detection of volatile gases

For volatile gas detection, the filter paper films were exposed to the HCl vapours and NH_3 vapours directly from their solution. The colour change of the filter paper was noted after successive exposure of HCl and NH_3 vapour.

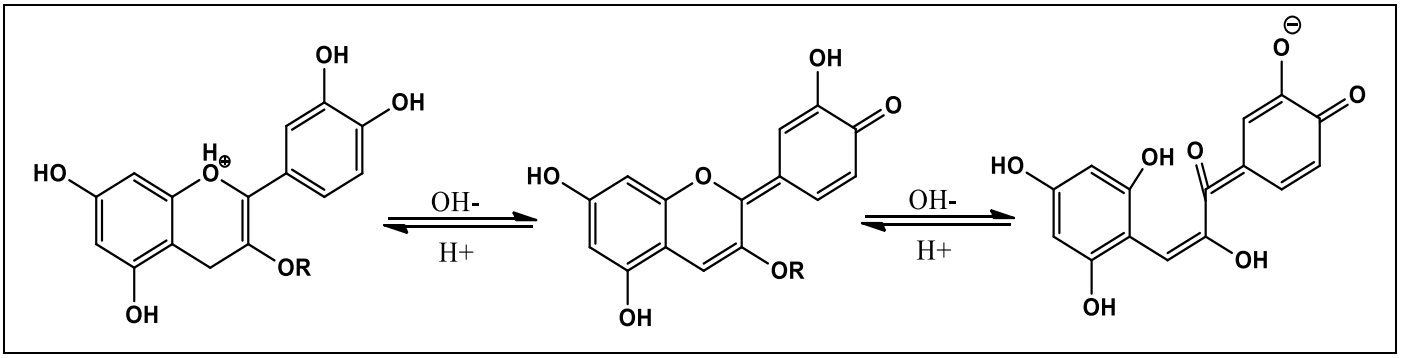
RESULTS AND DISCUSSION

Comparison of extracts of RC, VM, IF and AF

The potential of the anthocyanin-based extract prepared from various sources like RC, VM, IF and AF, as an intelligent dye, was verified through analysis of colour changes under different pH conditions (pH = 3, 5, 7, 9 and 11). The extract prepared from RC changed to pink at pH 3, pinkish violet at pH 5, blue at pH 7, green at pH 9 and yellow at pH 11 (Fig. 1). Similarly, VM shows the different colour under different pH solution. It shows slight pink at pH 3, violet at pH 5, blue at pH 7 and green at pH 9 and yellow at pH 11 (Fig. 2). The colour of IF and AF at various pH is demonstrated in the (Fig 1-2). (Table 1) summarizes the colour of various extracts at different pH solution.

The colour changes of anthocyanin-based extracts at different pH are evidenced from Fig.1 and Fig. 2 for various sources of extracts. The colour change might be due to the structural changes of anthocyanin structures as result of protonation and deprotonation reactions that interact with the sugars, bases and amino acids present along with the extract (no further purification of extract in the present investigation leading to the presence of amino acids and other extracts in extract solution) in many possible ways. Scheme 1 shows the schematic illustration of anthocyanin structure in acidic and basic medium. Since these extracts absorb the light in the visible region, they show appreciable colour change. Hence, RC, VM, IF and AF extracts could be used as pH indicator. On comparing the colour change in various pH of extracts, RC and AF showed predominant colour change in various pH. Similar results have been reported in extracts containing RC and rose extracts in the literature [Chen and Gu, 2013].

Pink colouration in acidic pH of the extract might be due to the exitance of flavylium cation. As the pH increases, quinone like structures of anthocyanins are formed and at extremely high pH ring opening reaction takes place. In the pH range of pH 5-7, equilibrium between quinone structures and flavylium structure might exist and hence violet or violet colour solution is observed at pH 5-7. Upon increasing the pH, quinonoid structure might be predominant in the pH range of 7-9 resulting in blue colour of the solution. Beyond pH 9, the colour turns into green or yellow due to the quinonoid open structure of anthocyanins.



Scheme 1. Schematic illustration of anthocyanin structure in acidic and basic pH

Table 1 Colour change of extract at various pH

S. No.	Source of Extract	Colour change				
		pH 5	pH 6	pH 7	pH 9	pH 11
1	RC	Pinkish Violet	Violet	Blue	Green	Yellow
2	VM	Pink	Pink	Bluish Pink	Light Green	Yellow
3	IF	Pink	Pinkish Violet	Blueish pink	Brown	Yellow
4	AF	Pink	Pinkish Violet	Blueish pink	Brown	Yellow

RC Extract

IF Extract

Fig 1 Photographic images of RC extract and IF extract at various pH solution (pH 5 - 11)

VM Extract

AF Extract

Fig 2 Photographic images of VM extract and AF extract at various pH solution (pH 5 - 11).

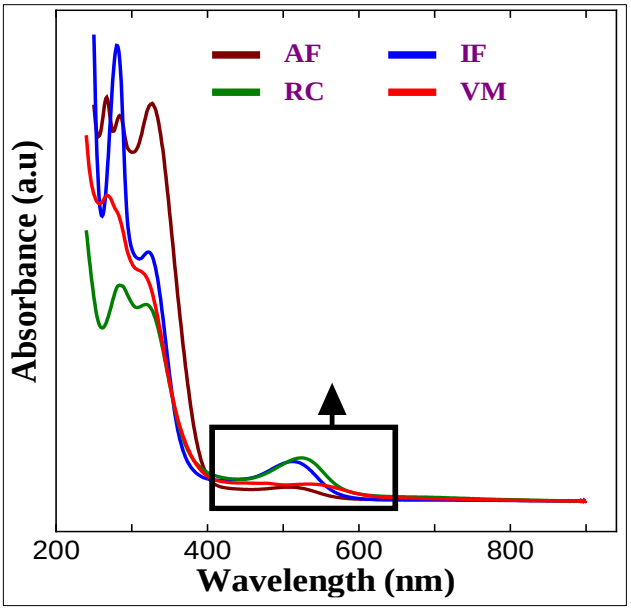
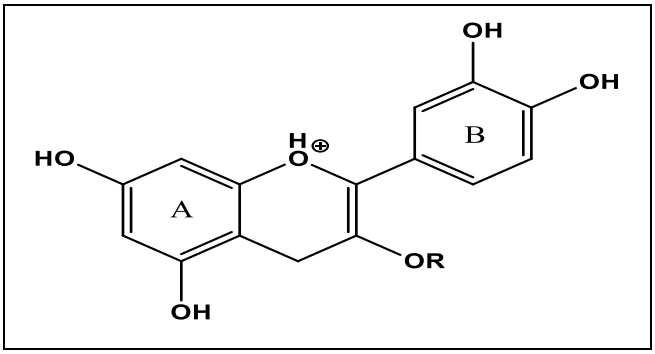


Fig 3 UV-visible spectrum of anthocyanins extracts from AF, IF, RC and VM at pH 3



Scheme 2 Structure of anthocyanins

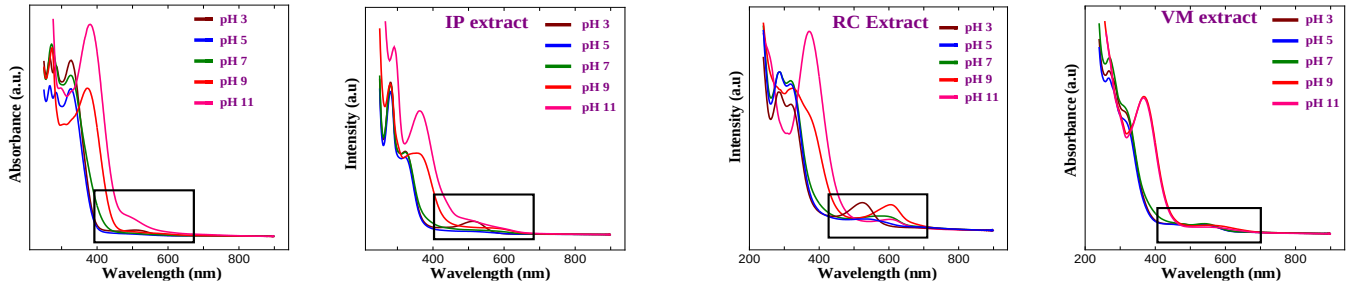
Characterization of extracts by UV-Visible spectroscopy

Generally, UV-vis spectrum of anthocyanins shows typical absorption band at around 500-530 nm, a weak shoulder peak in the range of 400 – 450 nm and peaks around 270 – 350 nm depending on the nature of anthocyanin and the substituents in it. It is evident from Fig. 3 that the anthocyanins present in all the four extracts are different or the substituents present in anthocyanin ring might be different. UV-vis spectra of IF and RC are almost similar showed a strong intense band at 513 nm and 524 nm, respectively due to the absorption band of ring B in scheme 2.

In general, the nature of substituents in ring B determines the colour of anthocyanin moiety. Increased hydroxyl substituents in ring B leads to bathochromic shift. In this present study, RC shows the maximum λmax value indicating the maximum hydroxyl substituents present in the extract compared to other extracts taken in the present investigation. Similarly, IF and AF also shows absorption maxima at 513 nm indicates the presence of hydroxyl functionalities in B ring. On the other hand, VM does not show only shoulder like band.

Comparison of UV-vis spectrum of extracts at various pH

To compare the activity of extracts, UV-visible spectra of extracts of AF, IF, RC and VM were recorded in the pH solutions of 3, 5, 7, 9 and 11. In the UV-visible spectrum of AF (Fig. 4) in pH 3 solution, a strong intense band was observed at 516 nm attributed to ring B in the anthocyanin structures in addition to then bands observed at 330 nm corresponding to ring A in the anthocyanin structure. Upon increasing the pH of the solution from 3 to 5, the band at 516 nm and 330 nm were decreased due to the structural change from flavylium cation to quinonoid structure as discussed in section 3.2. Further while increasing the pH from 5 to 7, the peaks at 516 nm completely vanishes and new shoulder peak start to appear at 524 nm. While increasing the pH from 7 to 9 increases the intensity of peak at 524 nm and a peak was observed at 371 nm and the peak at 330 nm completely disappeared. The same trend is also observed for pH 11 with much enhanced intensity. This might be due to the change of anthocyanin structure from quinonoid form into open chain structures. This clearly shows the pH dependent structural and UV-change of AF extracts. Similar trend is also observed for IF and VM. But the UV-spectral behaviour of RC is much different than that of AF, IF and VM.



AF Extract IP Extract RC Extract VM Extract

Fig 4 UV-visible spectra obtained for AF, IF, RC and VM extract at different pH solutions (pH 3, 5, 7, 9, 11)

In the case of RC extract, a strong intense band observed at 524 nm at pH 3 was red shifted to 537 nm as well as decrease in intensity at pH 5. In the meantime, the peaks observed at 330 nm and 301 nm at pH 3 were increased in intensity at pH 5. While increasing the pH from 5 to 7, the peak at 537 nm further red shifted to 590 nm and the intensity of peaks at 330 and 301 nm increases slightly compared to those observed for extracts at pH 5. Upon increasing the pH from 7 to 9, a new strong intense band was observed at 604 nm and the bands at 330 nm and 301 nm completely vanished and a new band was shoulder peak was observed at 375 nm might be due to the quinonoid form anthocyanin which is in equilibrium with open chain structure of anthocyanin. Further, increase in the pH from 9 to 11 shift the band of 604 nm into 608 nm with decreased intensity. In the meantime, the peak shoulder peak observed at 375 nm at pH 8 became very sharp at pH 11 with enhanced intensity. This might be due to the complete change of anthocyanin structure into open chain structure as indicated in Scheme 4.1. The pH dependent colour change of extracts as well as pH dependent UV spectra of RC makes it as better indicator for pH compared to other extracts like AF, IF and VM used in the present investigation. Hence, for further applications like volatile gas sensing and for monitoring the freshness of meat products, films prepared using RC was used in the present study.

Volatile gas sensors using RC coated filter paper

RC extracts were coated on normal filter paper using dip and dry method. For gas sensing the anthocyanin coated filter papers were exposed to volatile gases like HCl and NH_3. The extract coated filter papers were violet in colour. Being acidic nature of HCl vapours, it turned the anthocyanin coated filter paper into pink colour as evidenced from (Fig 5). While exposing the filter paper into NH_3 vapours, it turned the filter paper into green colour indicting the ability of anthocyanin coated films in sensing volatile gases like HCl and NH_3.

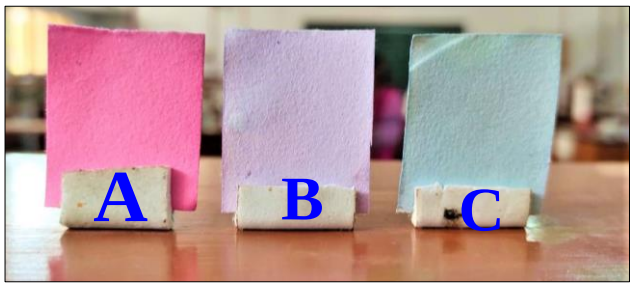


Fig 5 Anthocyanin coated filter paper (B) on filter paper (A) on exposing film B into HCl vapours and (C) exposing film B into NH_3 vapours

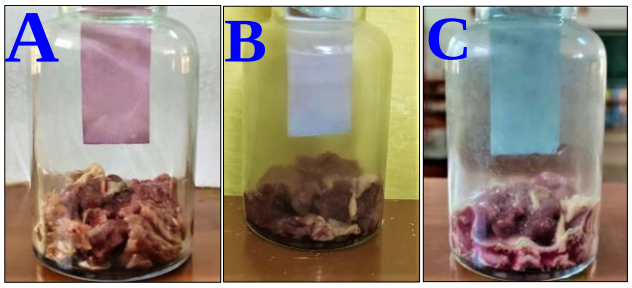
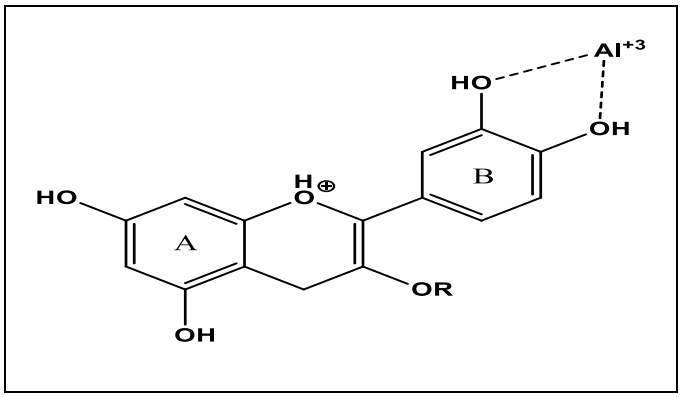


Fig. 6. Photographic images of monitoring the meat products using RC coated filter paper at (A) fresh meat products (B) after 4 h and (C) after 8 h

Monitoring the Freshness of Meat samples

Since meat products on decomposition produce ammonia by products, the decomposition of meat products can be monitored by sensing the ammonia vapours. It is evidenced from Fig. 5 that the anthocyanin extract coated filter paper is capable of sensing NH_3 vapours. Hence, RC extract coated filter paper was used for monitoring the freshness of meat and shrimp products purchased from local products. Fig. 6 shows the photographic images of monitoring of meat freshness using filter paper coated with RC extracts.

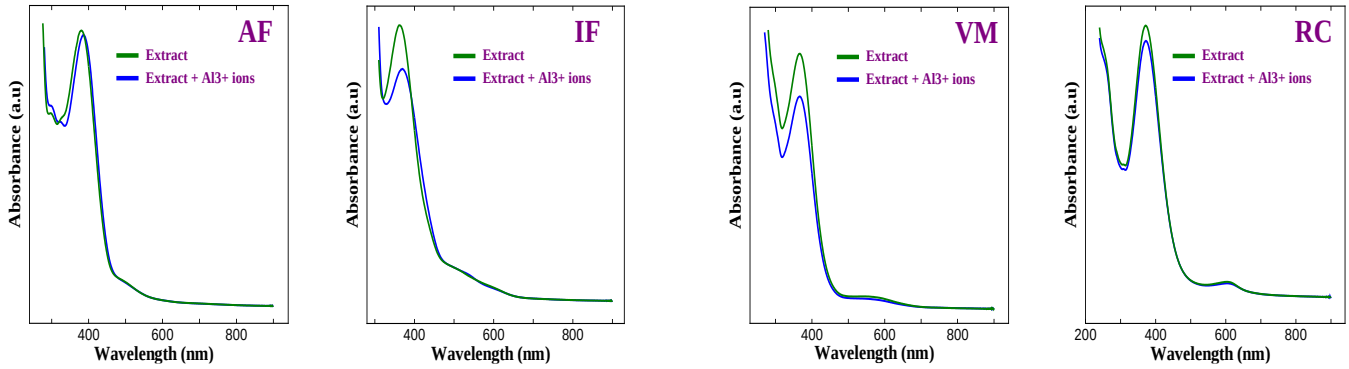
It is evidenced from Fig. 6 that the fresh meat products do not change the colour of RC coated filter paper. As the time progress, the decomposition meat takes place and the colour of filter paper start to change from pink to light blue in 4 h which completely changed into green at 8 h. This indicated that meat products completely decomposed within 8 h at room temperature which is indicated by the colour of filter paper. Hence, RC coated filter paper could be used for monitoring the freshness of meat products and possible to extend the applications in preparing smart packing materials using RC coated films for meat products.



Scheme 3 Complexation of anthocyanins with Al^{3+} ions

Interaction of anthocyanin extract with Al^{3+} ions

Further, the ability of anthocyanin extracts with Al^{3+} ions is also evaluated. It is well reported that the Al^{3+} ion has the greater tendency to form complex with anthocyanins due to the presence of hydroxyl functionalities present in ring B of anthocyanins (Scheme 3).



AF Extract IF Extract VM Extract RC Extract

Fig 7 UV-visible spectra obtained for AF, IF, RC and VM at pH 11 before and after the addition of 10^{-3} M Al^{3+} ions

The interaction of 10^{-3} M Al^{3+} with the AF, IF, RC and VM extract was analyzed at various pH 11 solutions since the complexation ability of anthocyanins predominant at basic pH due to the quinonoid structure of ring B at pH 11. Fig. 7 shows the UV-visible spectra obtained for extracts (AF, IF, RC and VM) at pH 11 before and after adding 10^{-3} M Al^{3+} ions. It is noted that the bands at ~530 nm are not affected by the addition of Al^{3+} ions. On the other hand, addition of Al^{3+} ions on extract at pH 11 not only decrease the intensity of the peak at 370 nm but also red shifted to a smaller extend. It is believed from the literature report that the Al^{3+} ions form the complex with quinonoid structure of ring B. Since the addition of Al^{3+} ions on the extract at basic pH decreased the intensity and red shifted the peak at 370 nm indicated that the peaks at 370 nm in basic pH of the extract might be due to the quinonoid form ring B in the structure of anthocyanin. Ring B peak of anthocyanin observed in acidic pH at 530 nm has been blue shifted to 370 nm due to the quinonoid structure of ring B as well as the open ring structure of anthocyanins. This reveals the ability of anthocyanin extracts to detect Al^{3+} in the basic pH.

CONCLUSION

In the present investigation, the ability of AF, IF, RC and VM extracts as pH indicators was compared to utilize them for various applications like monitoring the freshness of meat products and the visual volatile gas sensing of HCl and NH3. The extracts were prepared in aqueous solution by boiling the contents of AF, IF, RC and VM. The as-prepared extracts showed different colours at various pH solutions (pH 3 - 11). Further UV-visible spectra of all the extracts showed a sharp band at ~525 nm at acidic pH which disappeared and form a new band at 370 nm at basic pH. This might be due to the quinonoid structure formation as well as open chain structure of anthocyanin at basic pH. Since RC extract showed much clear colour change as well as well-defined UV-spectral bands at various pH, it was used for monitoring volatile gas sensing and monitoring the freshness of meat and shrimp samples. On exposing the RC extract coated filter paper on HCl and NH_3, it changed the colour from purple to pink and purple to green, respectively indicating the ability of RC coated films in sensing the volatile gases. Further, the RC coated filter paper was used to monitor the freshness of shrimp and meat samples. Both fresh samples of meat and shrimp do not change the colour of RC coated filter paper. The colour of filter paper slowly changed from purple to pink in meat and shrimp samples indicating the degradation of meat products. The colour change might be due to the formation of NH3 vapours due to the degradation of meat products. In addition, the extracts could also find applications in sensing Al3+ samples. After the interaction of Al3+ ions with the extract at pH 11, the intensity of peak at 370 nm was decreased and red shifted to slightly higher wavelength. This present investigation opens a new avenue for fabricating smart packing material to monitor the freshness of meat products constructing volatile gas sensor and to sense Al^{3+} metals ions in real sample.

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