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Development of a Betalain based biodegradable pH indicator film from beetroot peel for real time detection of chicken spoilage

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Abstract

Poultry meat is highly perishable and is a leading vehicle of bacterial foodborne illness, yet a large share of the food supply is discarded because freshness cannot be judged reliably from a printed use by date. This study reports the development of a low cost, biodegradable colorimetric pH indicator film that signals chicken spoilage in real time through a visible colour change. Betalain pigment, dominated by red violet betacyanins, was extracted in water from beetroot (*Beta vulgaris* L.) peel, an abundant agro processing byproduct. The pigment solution was cast into a flexible film using corn starch as the biopolymer matrix and glycerol as a plasticiser, with acetic acid as a mild acidifier, followed by gelatinisation at 65 °C and solvent casting on petriplates. The extract and the film showed clear, reproducible colour transitions from red through violet to yellow brown across the acidic to alkaline pH range relevant to meat spoilage. When applied to chicken stored at room temperature and under refrigeration, the film tracked spoilage over hours and days, respectively, changing colour as volatile nitrogen bases raised the surface pH. The film exhibited concentration dependent antioxidant activity (up to 55.6% at 50 mg/ml), inhibited *Escherichia coli* (25 mm zone) and had a mean thickness of 0.183 ± 0.003 mm. The results demonstrate that beetroot peel betalain in a corn starch matrix is a viable, non-toxic and sustainable platform for intelligent food packaging that valorises agricultural waste while improving food safety communication to consumers.

Keywords: betalain, betacyanin, beetroot peel, pH indicator film, corn starch, intelligent packaging, chicken freshness, biodegradable film

1. Introduction

Chicken is among the most widely consumed animal proteins worldwide, but it is also a leading source of bacterial foodborne illness. Salmonella and Campylobacter remain the principal pathogens associated with poultry and surveys indicate that roughly one in twenty five retail chicken packages can carry Salmonella. In parallel, an estimated nineteen percent of the food available to consumers is wasted globally, a loss driven in part by the difficulty of judging whether perishable products are still safe to eat. Conventional packaging communicates freshness only through a fixed, printed use by date that cannot account for temperature abuse, interrupted cold chains or variation between individual packages. There is therefore a clear need for packaging that reports the true, real time condition of the food it contains.

During poultry spoilage, microbial and enzymatic breakdown of proteins releases volatile basic compounds, collectively measured as total volatile basic nitrogen (TVB-N) and comprising ammonia, trimethylamine and other amines, which diffuse into the headspace and raise the local pH at the meat surface. Because this rise in pH is closely correlated with the progression of spoilage, a material that changes colour in response to pH can act as a simple, non-destructive and tag free freshness indicator that any consumer can read at a glance (Tahir *et al.*, 2022; Yong and Liu, 2023) [21, 23].

Natural pH responsive pigments are attractive for this purpose because they are non-toxic, biodegradable and derived from renewable sources. Anthocyanins and betalains are the two

most studied classes; betalains, in particular, provide vivid red violet betacyanins and yellow betaxanthins that undergo distinct, visible colour shifts across the meat relevant pH range and they retain good tinctorial strength at the low concentrations achievable from plant waste (Ndwandwe *et al.*, 2024; Abedi-Firoozjah *et al.*, 2023) ^[15, 1]. Beetroot (*Beta vulgaris* L.) is a rich and inexpensive source of betalains and its peel, normally discarded during processing, offers a route to valorise agricultural byproducts while supplying pigment for intelligent packaging (Setiawan *et al.*, 2015; Zin *et al.*, 2020) ^[17, 25].

To be useful, the pigment must be immobilised in a carrier film that is itself safe, flexible and environmentally benign. Biodegradable biopolymer matrices such as starch, chitosan, pectin and protein systems have all been used to host natural indicators (Ávila *et al.*, 2021; Roy and Rhim, 2021) ^[4, 16]. Corn starch is especially suitable: it is abundant, low cost, film forming and fully biodegradable and when plasticised with glycerol it yields a flexible, transparent film well matched to food contact applications, as shown by Basiak *et al.* (2019) ^[5]. The present work combines these ideas by developing a corn starch film loaded with beetroot peel betalain and evaluating its ability to indicate chicken spoilage in real time.

2. Materials and Methods

2.1 Raw materials

Fresh beetroot (*Beta vulgaris* L.) was procured from a local market and the peels, which are ordinarily discarded during processing, were separated, washed and used as the pigment source. Corn starch and food grade glycerol were used as the film forming biopolymer and plasticiser respectively and dilute (2%) acetic acid was used as a mild acidifier. Fresh chicken meat was obtained locally for the spoilage indication trials.

2.2 Extraction of betalain pigment

Betalain pigment was extracted from the beetroot peel into water. Cleaned peels were macerated with water to release the water soluble betacyanins and the coloured aqueous extract was separated from the solid residue by filtration. The pigment rich extract was collected and used directly as the aqueous phase for film casting; the pH and colour of the extracts were recorded prior to film formation (Kavas and Kavas, 2021) ^[12].

2.3 Preparation of the pH indicator film

The film was prepared by solvent casting. 100 millilitres of the pigment extracted water was taken, and 1.5 ml of glycerol together with 2 ml of 2% acetic acid was added and mixed well. Five grams of corn starch was then dispersed into the solution, which was heated in a water bath at 65 °C for 25 minutes until complete gelatinisation was achieved. 10 millilitres of the gelatinised solution were measured and poured onto a petri plate, left at room temperature for 24 hours to dry and the dehydrated film was then peeled from the plate. A control film was prepared identically using distilled water in place of the pigment extract (Elhrari *et al.*, 2025) ^[8].

2.4 Colorimetric (pH) response

The pH sensitivity of both the liquid extract and the cast film was assessed by exposure to a series of buffer solutions spanning the acidic to alkaline range. The colour of the extract and of the film was observed and recorded at each pH

value to establish the characteristic colour transition profile used to interpret spoilage (Ghanbar *et al.*, 2024) ^[10].

2.5 Application to chicken and spoilage monitoring

The indicator film was placed in contact with fresh chicken meat and two storage conditions were compared: samples held at room temperature and samples held under refrigeration. The colour of the film was monitored over time, in hours for the room temperature samples and in days for the refrigerated samples, alongside visual and organoleptic signs of spoilage, so that the film response could be related to the actual freshness state of the meat.

2.6 Antioxidant, antimicrobial and physical characterisation

2.6.1. Antioxidant activity (DPPH radical scavenging assay)

The extract and film samples were tested at multiple concentrations, following the classic method in which antioxidant activity is determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, following the original procedure described by Blois, 1958 ^[7].

2.6.2. Antimicrobial activity (disc diffusion method)

Antimicrobial activity against *S. aureus* and *E. coli* was evaluated using the standardized disc diffusion technique, based on the landmark method originally developed at the University of Washington, where paper disks impregnated with antimicrobial agents are placed on an agar plate seeded with the test organism, and the resulting zone of growth inhibition is measured after incubation to determine susceptibility (Bauer *et al.*, 1966) ^[6].

2.6.3. Film thickness measurement

Film thickness of both control and indicator films was measured using a digital micrometer at multiple points and reported as mean $\pm$ SD, following the standard method for tensile and physical testing of thin plastic sheeting.

3. Results and Discussion

3.1 Colour response of the extract and film to pH

Both the beetroot peel extract and the corn starch film incorporating it displayed clear, reproducible colour changes as the pH was varied across the range relevant to meat spoilage. Under acidic and near neutral conditions the pigment presented as bright to dark red, characteristic of protonated betacyanin, shifting towards violet and finally to yellow brown as conditions became alkaline. Because the colour transition is driven by the structural response of the betacyanin chromophore to pH, the film provides a direct visual scale that can be mapped onto freshness, with the red to violet and brown progression signalling advancing spoilage.

These observations agree with previously reported betalain systems. Roy and Rhim (2021) ^[16] found that films carrying beetroot extract shifted visibly from red toward yellow as the medium turned alkaline and used this transition to follow the freshness of chilled beef. A comparable red to violet to yellow sequence was reported for carboxymethyl cellulose and flaxseed gum films loaded with betacyanin from beetroot peel, which the authors described as a powerful colorimetric tool for beef freshness. Similar halochromic behaviour was documented by Nadi *et al.* (2023) ^[14] for anthocyanin based films made from red cabbage, confirming that pH driven colour change is a general and robust sensing mechanism for protein rich foods.

3.2 Chicken spoilage indication

When applied to chicken, the film tracked the two storage conditions as expected. At room temperature the meat spoiled within hours and the film changed colour over the same short timescale as volatile nitrogen bases accumulated and raised the surface pH. Under refrigeration, spoilage was delayed to a scale of days and the film changed colour correspondingly more slowly, mirroring the extended shelf life of the chilled product. The agreement between the colour change and the onset of spoilage under both regimes demonstrates that the film reports the real time condition of the meat rather than a fixed date.

This temperature dependent response is consistent with the wider literature on natural pigment freshness indicators. In a directly comparable study, a sago starch film containing betacyanin recovered from beetroot peel waste changed colour in step with the spoilage of packaged chicken, confirming beetroot peel betacyanin as an effective indicator for poultry specifically (Fadhallah *et al.*, 2023) ^[9]. Akhila *et al.* (2023) ^[2] likewise reported that a polyvinyl alcohol and guar gum film carrying a natural plant pigment monitored chicken freshness through a clear visual colour change during storage, and the systematic review of Tahir *et al.* (2022) ^[21] confirms that the colour response of such smart films correlates with measured TVB-N as spoilage advances.

3.3 pH of the extracts

The two beetroot peel extracts prepared for film casting were

mildly acidic, consistent with the stability window in which betacyanins retain their red colouration, as summarised in Table 1. Comparable mildly acidic pH and red to violet colouration for aqueous beetroot peel extracts have been reported by Setiawan *et al.* (2015) ^[17] and Zin *et al.* (2020) ^[25], who noted that betacyanins are most stable and most intensely red under weakly acidic conditions.

Table 1: pH and colour characteristics of the beetroot peel betalain extracts

Attribute	Extract 1	Extract 2
pH	5.96	5.0
Colour	Bright red to violet	Dark red to violet

3.4 Antioxidant activity

The film showed a clear concentration dependent antioxidant activity, rising from 13% at 10 mg/ml to 55.6% at 50 mg/ml (Figure 2). This radical scavenging capacity is attributable to the betalains and coextracted phenolics of the beetroot peel and is a valuable secondary function: an antioxidant active film can help retard oxidative deterioration of the packaged meat, giving the material an active as well as an intelligent role. A similar dose dependent increase in radical scavenging has been reported by Tahir *et al.* (2022) ^[21] and by Silva *et al.* (2020) ^[18] for betalain and anthocyanin rich extracts and films, where higher pigment content consistently raised antioxidant activity.

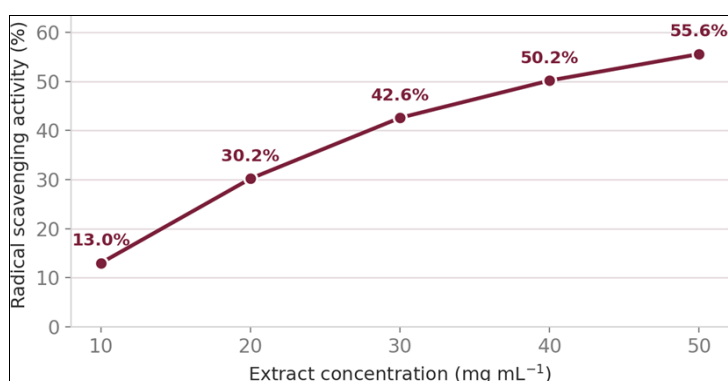


Fig 2: Antioxidant (radical scavenging) activity of the film

3.5 Antimicrobial activity

In the disc diffusion assay the film produced a 25 mm zone of inhibition against *Escherichia coli*, while no measurable inhibition was recorded against *Staphylococcus aureus* under the conditions tested (Figure 3). Activity against the Gram negative *E. coli*, a relevant indicator of poultry hygiene, indicates that the film contributes a degree of antimicrobial

protection in addition to its indicating and antioxidant functions. Betalain and beetroot derived films have similarly been reported by Soleiman Abadi *et al.* (2024) ^[19] to combine freshness indication with a food preservative, antibacterial effect that extended the shelf life of cold stored fish, supporting the use of such films as active intelligent packaging.

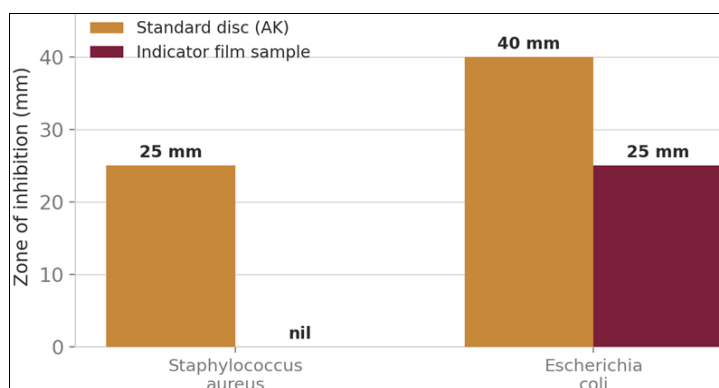


Fig 3: Zone of inhibition of the indicator film

3.6 Film thickness

Incorporating the pigment extract increased the mean film thickness from 0.150 ± 0.001 mm for the control to 0.183 ± 0.003 mm for the indicator film (Figure 4), reflecting the additional solids contributed by the extract. The values remain within the range typical of starch based food packaging films reported by Basiak *et al.* (2019) ^[15] and Yepes *et al.* (2018) ^[22] and are consistent with a flexible, handleable material.

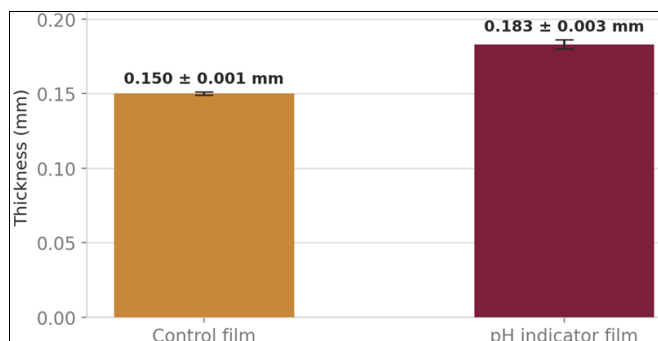


Fig 4: Mean thickness of the control and pH indicator films

3.7 Overall performance

Taken together, the results show that a single, simply prepared corn starch film loaded with beetroot peel betalain can perform three complementary roles: it reports meat freshness through a visible, pH driven colour change, it scavenges free radicals and it exerts antimicrobial activity against *E. coli*. The convergence of intelligent and active functions in a low cost, waste derived and biodegradable material is precisely what the recent reviews of Ndwandwe *et al.* (2024) ^[15] and Yong and Liu (2023) ^[23] identify as the direction of the field.

4. Conclusion

A natural, biodegradable pH indicator film was successfully developed using corn starch, glycerol and betalain extracted from beetroot peel, offering a promising solution for real-time detection of chicken spoilage. Corn starch served as the biopolymer and glycerol as the plasticiser, producing a flexible film suitable for food packaging, while the betacyanin-rich pigment supplied the pH-sensitive colour change. As chicken spoils, it releases volatile nitrogen compounds that raise pH and trigger a visible colour shift, allowing freshness to be judged directly. Using beetroot peel, an agricultural byproduct, also reduces food waste while providing a cost-effective natural pigment source.

The film offers an eco-friendly, non-toxic, inexpensive alternative to conventional plastic packaging, aligning with efforts to reduce plastic pollution and promote sustainable food packaging materials. Combining corn starch, glycerol and beetroot betalain into a single pH-sensitive film with added antioxidant and antimicrobial activity demonstrates a viable approach to monitoring chicken freshness. Future work should focus on correlating colour change with TVB-N and microbial load, improving pigment stability and water resistance and validating performance across broader storage conditions and packaging formats.

Conflict of Interest

Not available

Financial Support

Not available

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