



# Smart Packaging Approach: Development of Anthocyanin-Based Biodegradable Film for Milk Spoilage Detection

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**Abstract :** Anthocyanin-based biodegradable biosensor film was developed for real-time monitoring of milk spoilage detection. Anthocyanin pigment was extracted from hibiscus petals using 70% ethanol and incorporated into a 5% (w/v) corn starch matrix with glycerol (4–12 mL) as plasticizer via solution casting. The extract exhibited strong pH sensitivity across pH 1–14, showing a colour transition from red (acidic) to greenish-yellow (alkaline). The fabricated film retained similar pH-responsive behaviour with clear, visible colour changes. Phytochemical screening confirmed the presence of flavonoids, phenols, alkaloids, saponins, proteins, and organic acids. During milk spoilage, microbial activity produces lactic acid, lowering pH, which was effectively indicated by the film through rapid colour variation.

**IndexTerms -** Anthocyanin, Biosensor, Solvent Extraction, Phytochemicals, Smart Packaging.

## 1. INTRODUCTION

Milk is widely regarded as a “superfood” due to its exceptional nutritional profile and its ability to support overall health, growth, and development across all age groups. It is a naturally balanced food that provides all three essential macronutrients—proteins, fats, and carbohydrates—making it a complete dietary source. The proteins present in milk, primarily casein and whey, are of high biological value as they contain all essential amino acids required by the human body for tissue repair, muscle development, and enzymatic functions. In addition to proteins, milk is a rich source of easily digestible fats that provide energy and aid in the absorption of fat-soluble vitamins. Lactose, the primary carbohydrate in milk, serves as a quick energy source and also supports the growth of beneficial gut bacteria [1]. One of the most significant attributes of milk is its high calcium content, which plays a crucial role in the development and maintenance of strong bones and teeth, as well as in muscle contraction and nerve transmission. Milk also contains important vitamins such as vitamin A, which supports vision and immune function; vitamin B2 (riboflavin), which is essential for energy metabolism; vitamin B12, which is vital for red blood cell formation and neurological health; and vitamin D (in fortified milk), which enhances calcium absorption and bone health. Furthermore, milk provides essential minerals like phosphorus, potassium, and magnesium, contributing to bone strength, fluid balance, and enzymatic activity. Beyond basic nutrition, milk contains several bioactive compounds such as immunoglobulins, lactoferrin, and conjugated linoleic acid, which are known to enhance immunity and exhibit antimicrobial and potential anti-carcinogenic properties [2-3]. Its high-water content also makes it beneficial for hydration and maintaining electrolyte balance. Due to this combination of nutrients and functional components, milk plays a vital role in supporting growth in children, aiding muscle recovery in adults, and preventing bone-related disorders in the elderly. However, despite its numerous health benefits, milk may not be suitable for everyone, as some individuals may experience lactose intolerance or milk allergies, and excessive consumption of high-fat milk may pose risks for certain health conditions. Overall, milk’s comprehensive nutritional composition, functional benefits, and accessibility justify its classification as a superfood, while also highlighting its importance in both dietary nutrition and food science research, particularly in areas such as spoilage detection and smart packaging systems [4-6].

Although milk being superfood, consumption of spoiled milk can have several harmful effects on the human body due to the presence of harmful bacteria and toxins produced during microbial activity. It can lead to food poisoning, which commonly causes symptoms such as stomach cramps, nausea, vomiting, and diarrhoea. In more serious cases, it may also result in fever, dehydration, and weakness. Children, elderly individuals, and people with weak immune systems are more vulnerable and may experience stronger reactions. Continuous or repeated consumption of contaminated milk can also disturb the digestive system and negatively affect gut health over time [5].

The most common method people use to detect milk spoilage is smell, where a sour or unpleasant odor is taken as a sign of spoilage, but this can vary from person to person since sensitivity to smell is different for everyone and can even be affected by health or surroundings [2].

Apart from these commonly used methods, there are a few other ways that people or small dairy setups sometimes use to check spoilage, but they also have their own limitations. One of these is checking the acidity level of milk. As milk begins to spoil, bacteria produce lactic acid which increases its acidity. In some cases, litmus paper is used to observe this change, where a Color shift can give a rough idea about the condition of the milk. However, this method is not very common in households and does not clearly indicate whether the milk is safe to consume.

In certain situations, an alcohol test is also used, especially in small dairy practices. However, this method requires proper materials and is not practical for everyday household use. Detection using a *pH* sensitive film offers a simple and practical way to identify the spoilage of milk and curd without depending on guesswork or unsafe methods [7-10].

Thus, this research is an approach in the direction offering a smart bio packaging solution to develop Anthocyanin-Based Biodegradable film for milk spoilage detection.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Hibiscus flower and Corn Starch were procured locally, near from the institute (Neori, Vikas, Ranchi, Jharkhand), Distilled Water (D/W), Ethanol, Ethyl Acetate, Glycerol, FeCl<sub>3</sub>, NaOH, HCl, Iodine, KI, Phenolphthalein, Chloroform were procured from Sisco Research Laboratories Pvt. Ltd. Maharashtra.

### 2.2. Methods

#### 2.2.1. Extraction of Bio-pigments

Fresh flower sample was collected locally and washed properly. After washing, the petals were separated and fine chopping was done to cut into small pieces. 70% ethanol, D/W, ethyl acetate was used as a solvent for anthocyanin pigmentation. The best extraction was observed in 70% ethanol solution after heating at 50-60 °C for 30 minutes. The extract was collected by filtration.

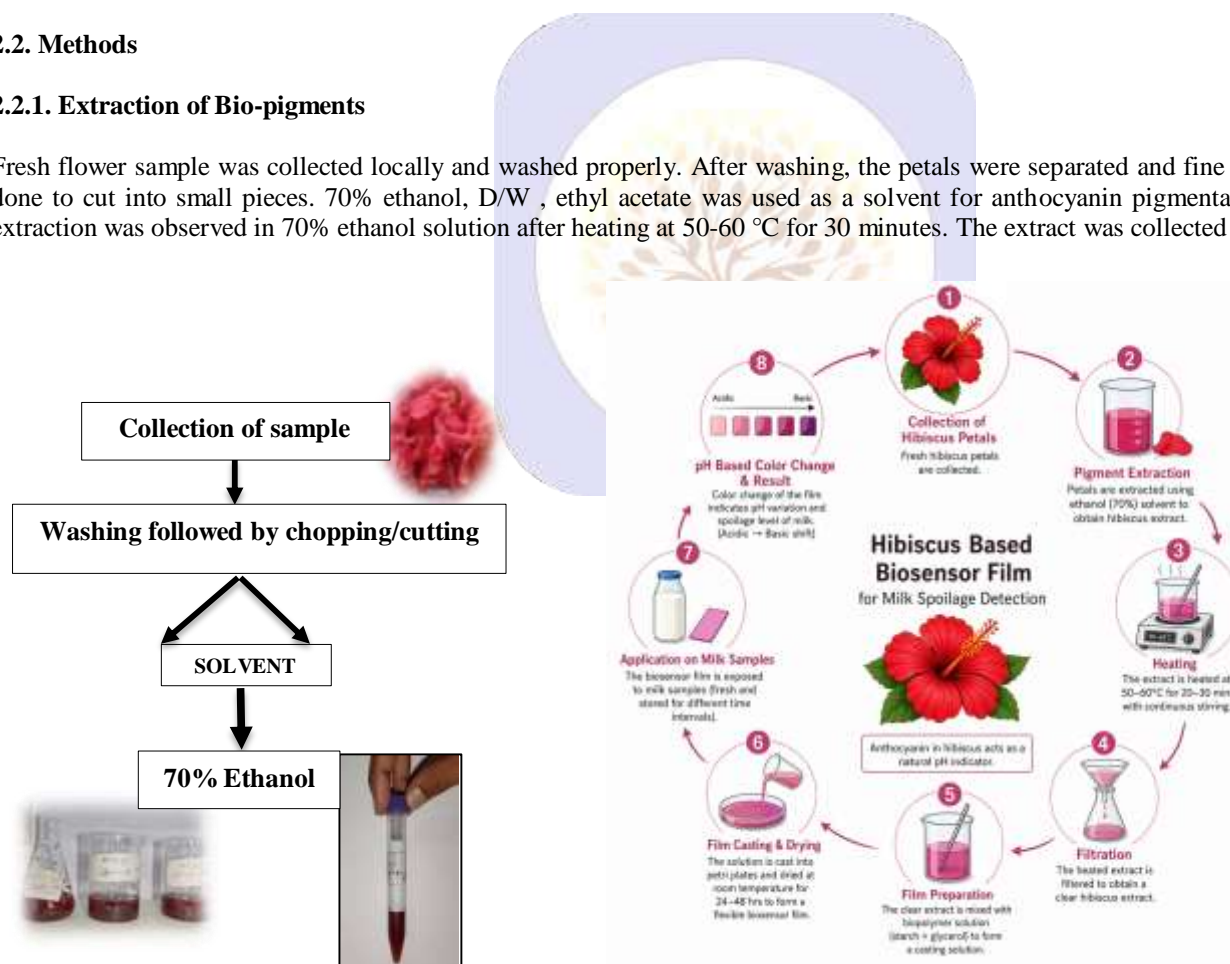


Figure 1: Extraction of Anthocyanin Pigment

#### 2.2.2. Development of Anthocyanin-Based Biodegradable Film

5 % starch solution was made by dissolving 5 g of corn starch in 100ml of distilled water followed by heating at 80°C for approx. 1 hr, until a viscous solution was obtained. Glycerol as plasticizer was added slowly to the starch solution and mixed thoroughly with the help of glass rod. Different concentration of glycerol was added to the different set of solutions for the standardization process to obtain a desired biosensor film. Further, anthocyanin extract was further added in the solution to form pH sensitive film. The prepared solutions were then poured and cast into the petri plates for the film formation (Figure 2).



Figure 2: Biosensor Films

Table 1: Synthesis and optimization of biosensor

S.N.	CONCENTRATION OF CORN STARCH (W/V)	GLYCEROL
1.	5%	4ml
2.	5%	6ml
3.	5%	8ml
4.	5%	10ml
5.	5%	12ml

2.2.3. Phytochemical analysis of the extract

Table 2: Phytochemical Analysis

S.N.	PHYTOCHEMICAL	PRESENT/ABSENT
1.	Phenol	Present
2.	Flavonoids	Present
3.	Saponins	Present
4.	Alkaloids	Present
5.	Protein	Present
6.	Organic acid	Present

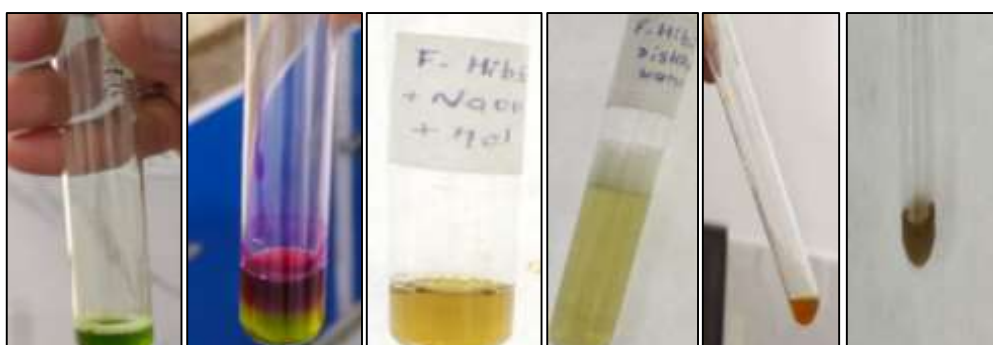


Figure 3: Phytochemical analysis of Anthocyanin pigment

2.2.4. pH sensitivity Analysis of the Extract

**Preparation of standard pH solutions:** Concentrated HCL and NaOH was used for the preparation of known strength of standard pH solutions. A strong acidic solution of pH 1 and strong basic solution of pH 14 was prepared first and rest of the solutions from pH 2 to pH 13 was prepared by serial dilution method. The prepared standard pH solutions were then used for the initial pH sensitivity analysis of the anthocyanin pH sensitivity.



Figure 4: *pH* sensitivity of anthocyanin pigment extract

### 3. CONCLUSION

Anthocyanin pigment was successfully extracted from the hibiscus petals collected locally near the institute. Out of the three solvents, the best extraction was observed by 70% ethanol. The pigment showed a very good sensitivity against the range of standard *pH* solutions from *pH* 1 to *pH* 14. A deep red colour was observed at highly acidic *pH* whereas, it was observed to be prominent yellowish green due to protonation and deprotonation of anthocyanin molecule. Also, anthocyanin fabricated starch-based film also has the similar result and can be used a smart packaging solution for milk spoilage detection.

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