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Fabrication of Curcumin integrated Biodegradable Biosensor Film for Real-Time Monitoring of Milk Freshness

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Abstract : The present study aims to develop a pH-sensitive biosensor film using natural pigment and to evaluate its effectiveness in detecting milk spoilage in a safe, simple, and reliable manner. A biosensor film based on natural pH indicators was developed using plant-derived pigment. Turmeric powder, containing curcumin, was selected due to its known ability to exhibit visible colour changes in response to variations in pH. The pigments were extracted using different solvents under controlled heating conditions to ensure efficient recovery of bioactive compounds without degradation. The filtered extracts were then combined to enhance the sensitivity and range of colour response.

Corn starch-based biopolymer matrix was prepared using distilled water, followed by heating to induce gelatinization and form a uniform viscous solution.

To evaluate the performance of the developed film, a series of standard pH solutions ranging from pH 1 to pH 14 were prepared using hydrochloric acid and sodium hydroxide through serial dilution. The biosensor film was exposed to these solutions to analyze its color-changing behaviour across a wide pH range. The results demonstrated clear and distinguishable color variations corresponding to different levels of acidity and basicity, confirming the film's sensitivity and effectiveness as a pH indicator.

***IndexTerms* - Biosensor, smart-packaging, pH sensitive, Curcumin**

1. INTRODUCTION

Milk is widely regarded as a natural "superfood" because of its balanced nutritional composition and its role in supporting human life from infancy to adulthood. It serves as the first complete source of nutrition for newborns and continues to provide essential nutrients such as calcium, phosphorus, proteins, and vitamins that contribute to bone strength, muscle development, and overall health. In addition to direct consumption, milk is also processed into common dietary products like curd, paneer, butter, and ghee, making it an integral part of everyday nutrition [1].

However, milk is highly perishable and prone to spoilage due to the rapid growth of microorganisms, particularly bacteria. These microbes can enter milk during handling, storage, or from the environment and multiply quickly under favourable conditions, especially at room temperature. As they break down lactose into lactic acid, the milk becomes increasingly acidic, leading to sour taste, unpleasant odour, and changes in texture such as curdling and separation. Spoilage may begin even before visible signs appear, making early detection difficult [2].

Consumption of spoiled milk can pose serious health risks, including food poisoning, which may result in symptoms like nausea, vomiting, diarrhoea, stomach cramps, and in severe cases, fever and dehydration [3]. Vulnerable groups such as children, the elderly, and individuals with weakened immunity are particularly at risk. Additionally, toxins produced by certain bacteria can harm the digestive system and overall gut health [4-6].

Current methods for detecting milk spoilage largely depend on sensory evaluation, such as smell and visual inspection. However, these methods are subjective, inconsistent, and often detect spoilage only after it has significantly progressed. This limitation highlights the need for a more reliable, early-stage detection system [7-8].

To address this challenge, the present research proposes the development of a biodegradable biosensor film for real-time monitoring of milk freshness. The film is prepared using corn-based starch as the biopolymer matrix, combined with natural pH-sensitive pigments extracted from turmeric. These pigments, containing curcumin respectively, respond to changes in pH by exhibiting visible color changes [4]. As milk spoils and its acidity increases, the biosensor film changes color, providing a simple, safe, and effective visual indicator of freshness. This approach offers a sustainable and practical solution for early detection of milk spoilage, improving food safety and consumer awareness.

2. MATERIALS AND METHODS

2.1 Materials

Turmeric and Corn Starch were procured locally, near the institute (Neori, Vikas, Ranchi, Jharkhand), Distilled Water (D/W), Ethanol, Ethyl Acetate, Glycerol, $FeCl_3$, 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

Three different solvents—70% ethanol, ethyl acetate, and distilled water—were used for extraction. Each solvent was added to the respective containers so that the samples were fully soaked. The mixtures were stirred gently to mix well.

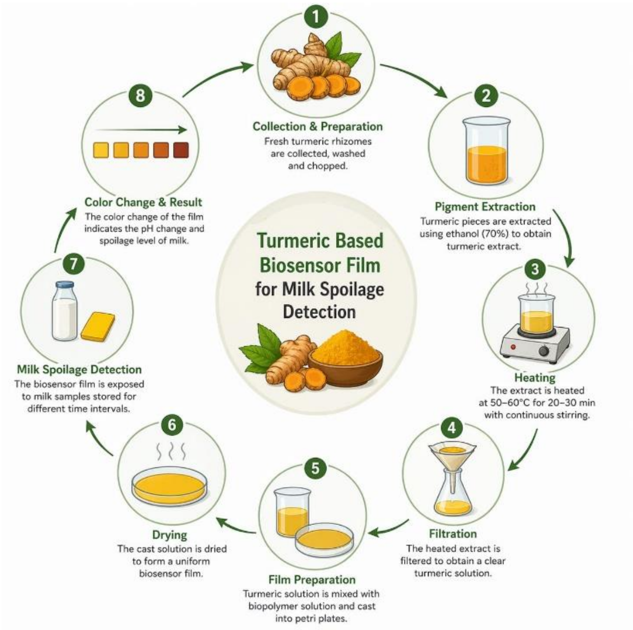

Figure 1: Extraction and preparation of Bio pigment-based biosensor film

These mixtures were then heated on a hot plate at around 50–60°C for about 20–30 minutes with continuous stirring. This helped in better extraction of pigments, while care was taken not to overheat and damage the natural compounds. A visible color change during heating showed that extraction was happening successfully.
After heating, the mixtures were allowed to cool naturally. They were then filtered to remove solid particles, leaving behind clear liquid extracts. These extracts contained natural pigments—anthocyanins from hibiscus and curcumin from turmeric.
Finally, turmeric extract was stored in a clean, airtight container to avoid contamination. This solution was then ready to be used for making the biosensor film.

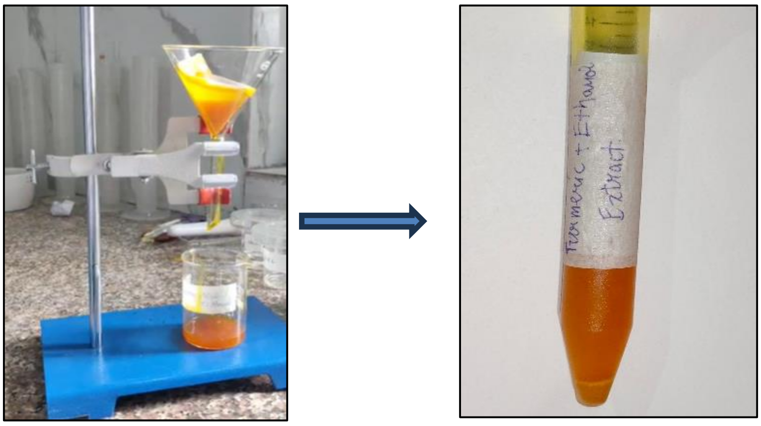

Figure 2: Bio-Pigment extraction

2.2.2 Preparation of Biosensor Film

5g of corn starch was dissolved in 80ml of D/W and allowed to heat at 80°C for approx. for 120min until a viscous solution was obtained followed by addition of glycerol. Different concentration of glycerol was added to obtain a desired biosensor film. The extract was further added to the solution. The prepared solution was then poured and cast into the petri plates for the film formation **(Figure 3).**

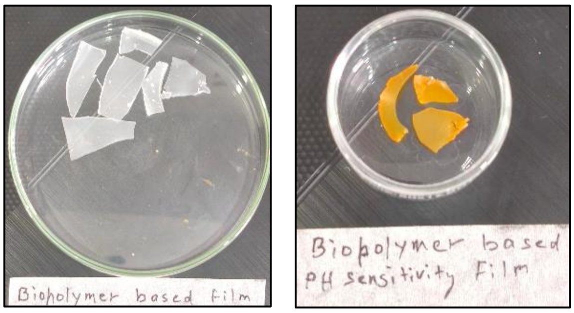

Figure 3: Biosensor Films

Table 1: Synthesis and Optimization of film

S.N.	CONCENTRATION OF STARCH (gm)	GLYCEROL
1.	5g	4ml
2.	5g	6ml
3.	5g	8ml
4.	5g	10ml
5.	5g	12ml

2.2.3 Phytochemical Analysis of the Extracts

Phytochemicals of the extract were analysed using various reagent tests. The presence of different phytochemicals in the extract makes them suitable for the biosensor film development and confirms the pH sensitivity.

Table 2: Phytochemical analysis of extract

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

2.2.4 pH Sensitivity Analysis of the Extract

2.2.4.1 Preparation of standard pH solutions

Standard pH solutions were prepared to test how the biosensor film responds to different levels of acidity and basicity. hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used because they easily dissolve in water and provide controlled pH levels. A strongly acidic solution of pH 1 was first prepared using HCl as stock solution, and then gradually diluted step by step (serial dilution) to obtain solutions with increasing pH (less acidic). Similarly, a strong basic solution of pH 14 was made using NaOH as stock solution and then diluted to get solutions with decreasing basic strength.

This process resulted in a complete pH range from 1 to 14. These solutions were then used to test initially for the extracts followed by the biosensor film, helping to observe its Color changes at different pH levels and evaluate how effectively it can detect acidity changes like those that occur during milk spoilage.

2.2.4.2 pH sensitivity analysis

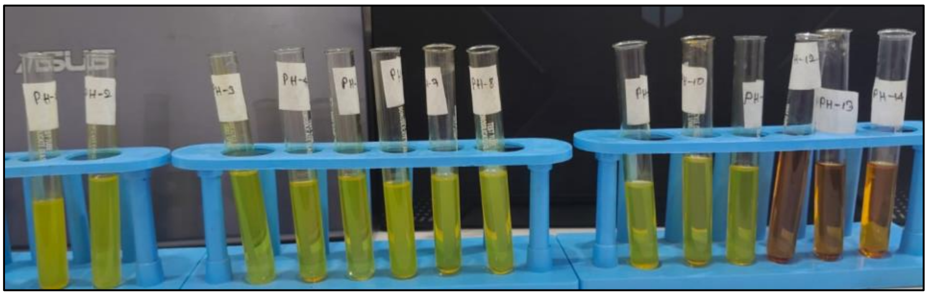

Figure 4: pH sensitivity of turmeric extract

3. CONCLUSION

Bio pigment curcumin was successfully extracted from Turmeric powder. Using solvent extraction method. Out of the various solvent used, the best extraction was with 70% ethanol due to the pigment solubility. The film formation was successfully achieved by corn base starch using glycerol as plasticizer. The pH sensitive bio-sensor film developed using curcumin showed a good pH sensitivity across the various range from pH 1 to pH 14. The color of the film was observed to be yellow in acidic pH where as it was reddish brown to orange in the basic pH due to protonation and deprotonation respective medium (**Figure 3).**

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