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Optical Biosensor for the Detection of Hydrogen Peroxide in Milk

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Abstract: Over the years, the food industry's concern to provide safe food that does not cause harm or illness to consumers has increased. The growing demand and detection of compounds that can contaminate food is increasingly demanding. Hydrogen peroxide is frequently used as a substance to control the growth of microorganisms in milk, thus increasing its shelf life. Here is presented a strategy for the detection of hydrogen peroxide as a milk adulterant, using a single shot membrane sensor. The lowest concentration measured with this technique was 0.002% w/w of H_2O_2, in semi-fat milk.

Keywords: Chemiluminescence; Hydrogen peroxide; Optical sensor; Food safety; Food fraud; Quality assessment

1. Introduction

Milk is one of the most complete foods for humans, containing nutrients including carbohydrates, proteins, fats, minerals, and vitamins [1].

Owing to its rich composition, milk becomes a substrate for the growth of undesirable microorganisms that can easily deteriorate milk. To prevent this from happening, prohibited substances are fraudulently added [2]. Hydrogen peroxide (H_2O_2), hypochlorite, formaldehyde, potassium dichromate and salicylic acid are examples of substances used as adulterants that need monitoring and quality control as they are toxic to humans [3].

In the case of H_2O_2, it is widely used in the dairy industry as an antimicrobial agent, thus helping preserve the raw milk in the absence of refrigeration [4]. Despite its conventional use, when added to milk, H_2O_2 can cause a decrease in the nutritional value of the food, due to the destruction of vitamins A and E, which generates reactive and cytotoxic oxygen species, including hydroxyl radicals, that can initiate oxidation and damage nucleic acids, lipids and proteins. Consequently, when ingested, milk can lead to negative effects on the health of the population, especially in immunocompromised people [2][4].

In the USA, hydrogen peroxide is used in cheese production in concentrations up to 0.05% w/w, however in other countries its addition is prohibited due to its toxic effects. Peroxide concentration > 0.1% w/w induce cancer in the duodenum of mice and present short-term genotoxicity [3].

Here, it is presented a study for the detection and quantification of H_2O_2 using a chemiluminescence technique. A small low-cost hydroxyethyl cellulose sensitive

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membrane combined with a high-sensitive photodetector is used to measure H_2O_2 concentrations in semi-fat milk samples.

2. Materials and Methods

The sensing methodology is based on the detection of a luminescence signal from the chemical reaction within a solid membrane produced with hydroxyethyl cellulose (HEC, Sigma Aldrich, Germany), luminol, sodium phosphate, cobalt (II) chloride hexahydrate, sodium lauryl sulphate (SLS) and ethylenediaminetetraacetic acid (EDTA).

The procedure established by Omanovic-Miklicanin and Valzacchi 2017 was refined to establish the experimental protocols. For the determination of H_2O_2 in very low concentrations, the sensor sensitivity should be as high as possible. Therefore, systematic optimization of the membrane was necessary. Only one constituent was varied at a time, keeping the remaining constituents unchanged. After membrane optimization the final concentrations of these constituents were set to luminol (0.2 mg), sodium phosphate (8.6 mg), SLS (60 μL, 34.36 mmol/L), cobalt hydroxide (100μL, 5.0 mmol/L), EDTA (2 μL, 20 μmol/L) and HEC (150 mg) was added to 10 ml of Milli-Q® water.

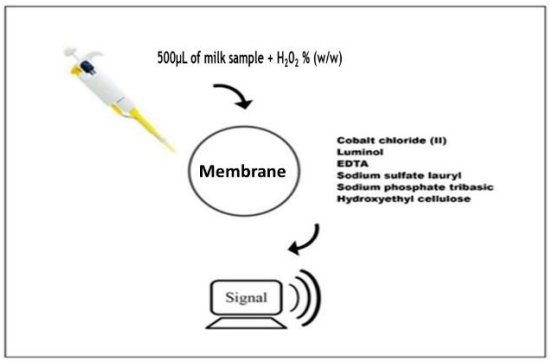
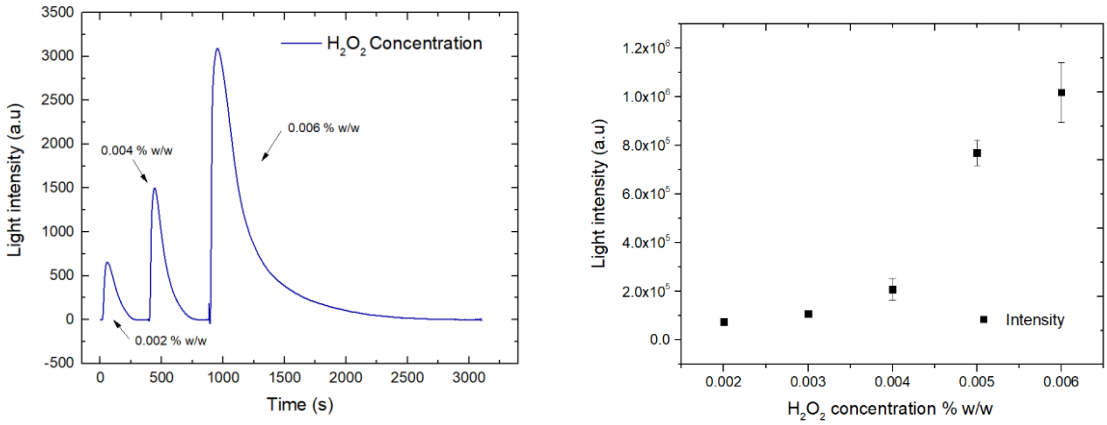


Figure 1. : Schematic diagram of the analyte detection.

The membrane solution was placed on a magnetic stirrer for 30 minutes. Individual 3D printed cups were used and 1000 μL of membrane solution was added and dried for 4 hours (T = 70 ºC). After drying, the membranes were stored in a desiccator under vacuum. For the measurement procedure, the membrane was placed directly onto the membrane holder on top of the detector, which was specially designed to have high sensitivity and different gains. The light emission was measured by adding 500 μL of the sample solution as shown in Figure 1.

3. Results and Discussion

Semi-fat milk samples were adulterated with H_20_2 concentrations from 0.001% w/w to 0.006% w/w by diluting a standard 30% w/w solution of H_2O_2. The variation of the fluorescence intensity is presented in Figure 2 for all samples, together with the time integral of the decaying fluorescence signal for each H_2O_2 concentration.



(**a**) (**b**)

Figure 2.: (**a**) Variation of the intensity of the light emission for the concentrations of 0.002, 0.004 and 0.006 %w/w as a function of time; (**b**) Time integral of the decay time for each H_2O_2 concentration.

Taking into consideration that 0.05 % w/w of H_2O_2 is the defined limit for the FDA in milk for cheese production [6], the developed sensor would be suitable for determinations of H_2O_2 as a fraud controller in milk samples, within the legal limits of different countries. Moreover, to achieve a more practical approach to the commonly time-consuming sample preparation methods, the pre-treatment step was successfully eliminated. In fact, the optimized sensor requires minimal solvent use and waste production. Besides, when compared with other methods available for the determination of H_2O_2 presence in milk, this portable biosensor is an easy and reliable method that ensures the required sensitivity, while offering a low time of analysis, and no need for additional laboratory equipment.

The methodology developed and optimized, demonstrates that is possible to detect very low concentrations of H_2O_2 (down to 0.001 % w/w in an aqueous system). As the H_2O_2 concentration increased, the intensity of the emitted light and the reaction time increased. Low limits of detection were achieved, thus indicating the applicability of this essay to real samples exhibiting the required sensitivity for the analytical determination of H_2O_2 in biological samples such as milk.

4. Conclusions

The proposed sensor provided to be a rapid, cost-effective, and environmentally friendly approach for the determination of hydrogen peroxide as a milk adulterant. This optimized and validated method has a very good linearity range when the sample is in its liquid state, where concentrations of H_2O_2 as low as 0.001% w/w can be detected with good repeatability. As a practical application for this methodology under controlled conditions, an adulterated milk sample was analyzed. Concentrations of H_2O_2 of 0.002% w/w to 0.006% were detected and method was calibrated for semi-fat milk, proven that limit of detection and linearity range of the proposed method are suitable for the analysis of milk samples in loco, which can add value to the food fraud department. Moreover, the reagents required are commonly used in analytical laboratories, inexpensive, and consumed in low amounts (500 µL), thus resulting in negligible and non-toxic waste generation. In addition to the mentioned advantageous features, the proposed method validation is comparable to those found in the literature.

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Conflicts of Interest: The authors declare no conflict of interest.

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