Detection of gelatin in ice cream using QCM sensor

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Abstract. Gelatin in ice cream samples has been successfully identified using Quartz Crystal Microbalance (QCM) sensors. The sensor was prepared by polyaniline and nickel nanoparticles modification. The modification was deposited on the surface of QCM gold electrode using layer by layer (LbL) method. Performance of the sensor was investigated for gelatin determination in the homemade ice cream samples. The homemade ice cream was made with and without porcine or bovine gelatins addition. The experiments were performed in various concentration of the ice cream (100, 200, 300, 400, and 500 ppm) at pH 9. The measurement was compared to the standard of porcine and bovine gelatins. The results showed that the sensor can be identified gelatin in ice cream samples. Frequency shift of ice cream that contained porcine gelatin was appeared as positive value, whereas a negative shift value was occurred for ice cream that contained bovine gelatin.

1. Introduction
Gelatin is a water-soluble protein which is partial hydrolysis of collagen from the skin, bones, and connective tissue of animals [1]. Gelatin has properties similar to collagen because of its softness and elasticity. The formation of gel is reversible depending on temperature [2]. Gelatin contains several amino acids in large quantities such as glycine, proline, and hydroxyproline. Hydrocolloid hydrolyzate found in gelatin is widely used for making jelly and various foods [3]. The ability of gelatin to form gel makes gelatin widely used in food & beverage industries pharmaceutical industry, beauty, chemical industry and military [4]. In food industries, gelatin functions as an emulsifier, gelling agent, thickener, setting agent, clarifying agent, water-retaining agent and stabilizer in manufacturing marshmallow, meat processing, candy, jelly, and ice cream [5].

One product that contains gelatin which is currently the most sought after by consumers from children to adults is ice cream. Based on a London Psychiatric Institute survey, England [6], some volunteers claimed to be more comfortable after consuming ice cream. Articles such as marketeers.com stated that according to Euromonitor, the Indonesian ice cream market is very large, reaching 158 million liters and its largest in Southeast Asia. By 2018, the growth of the ice cream market will reach 240 million liters equal to average growth of 8.75%. Still according to Euromonitor data, the Asia Pacific Region controls the largest ice cream market around 30% of the total world ice
cream market. Significant use causes gelatin to be produced in large quantities. Based on the survey that has been done, gelatin production reaches 326,000 tons annually, with raw materials of pig skin (46%), cattle (29.4%), bovine (23.1%), and other raw materials (1.5%) [2]. The large amount of gelatin produced from pigs makes some groups of people such as Muslims and Jewish, who are not allowed to consume pigs, want to label the type of gelatin used in which is the products [5]. In this case, fish and beef gelatin has possibility as alternative to be used because it is acceptable product for Islam, Judaism and Hinduism with minimal restrictions [1].

Various sources of gelatin are an important issue for the Muslim community because of their awareness of halal food. Halal Muslim (purification) law requires all types of products (both food and non-food) that are free of pigs and their derivatives [7]. Currently, Muslim consumers rely on certification and labeling ensuring that the products used are produced by the halal production process [8] because these "halal" products are not easily verified by ordinary persons [9]. For example, in the case of gelatin, the similar physical appearance of cow and pig gelatin makes both of them indistinguishable. Therefore, more research was carried out using various methods to distinguish cow and pork gelatin.

Several studies have been reported regarding the methods that can be carried out to detect the type of gelatin, both bovine and porcine gelatin. The precipitation method to identify the type of gelatin was reported by Hidaka and Liu[10]. More selective detection of gelatin is done by protein identification. Some methods such as Polymerase Chain Reaction (PCR) by [11][12], Enzyme-linked immunosorbent assay (ELISA) [13] and High Performance Liquid Chromatography-Mass Spectroscopy (HPLC-MS) [14]. Gelatin sources can be distinguished using the HPLC-MS method [15]. The mass spectrum obtained was compared to collagen database available. Fourier Transform Infrared (FTIR) spectroscopy method has also been carried out [16]. Data generated from this method did not provide a significant difference. Other researchers have also conducted experiments on the detection of pig and cow gelatin on marshmallow samples using a QCM sensor. The results showed that increasing frequency was observed on marshmallow samples containing pork gelatin, whereas marshmallow samples containing cow gelatin produced decreasing frequency [17].

The QCM sensor has been developed because it has high sensitivity and can be used at room temperature. In addition, the operation of sensor is also easy, fast and can be monitored directly (real time analysis) [18]. QCM sensor shows good sensitivity performance for both gas and liquid phases. When the analyte target is attached to the surface of the QCM coating (adsorbent), the mass will increase. As a result, the resonant frequency changes [19]. Based on these explanations, in this study, we made QCM sensors by polyaniline/ nickel compounds modification using a layer by layer deposition technique (LbL) technique to detect gelatin sources in ice cream samples.

2. Methods

2.1. Materials

Sodium hydroxide [NaOH], hydrochloric acid [HCl], and aniline [C₆H₅NH₂] were purchased from Merck. Porcine and bovine gelatins were purchased from local store in Surabaya, East Java, Indonesia. Quartz Crystal Microbalance (QCM) used a commercial 5 MHz AT-cut quartz crystal (diameter 25.4 mm) which was purchased from Renlux Crystal (Shenzhen, China). Nickel compound was synthesized based on explained methods in our previous work [20]. Demineralized water was used for all cleaning, preparation, and measurement.

2.2. Modification of the QCM sensor

The gold QCM sensor that has been treated as mentioned above was electropolymerized in 0.1 M aniline solution at pH 1.5 (the pH was adjusted using HCl solution). The electropolymerization was conducted using cyclic voltammetry technique with three-electrode cell system, which gold QCM sensor as working electrode (WE), Ag/AgCl (3M KCl) as reference electrode (RE), and platinum as counter electrode (CE). It was carried out at scan rate of 50 mV/s over potential -0.5V to 1.0 V for forty cycles. The deposition of nickel compound on the surface of the working electrode [20] was
conducted using Layer by Layer (LbL) deposition technique [21]. Briefly, the QCM sensor was immersed in nickel solution for 15 min then dried in room temperature.

2.3. Sample preparation
2.3.1. Ice cream production. There were two kinds of ice cream to be made; the first ice cream will contain bovine gelatin while the second ice cream will contain pork gelatin. 75 grams of ‘Pondan’ ice cream powder (composition: milk, sugar, gelatin, coconut milk, eggs) was mixed with 150 mL of ice water. The mixture was beat for 5 minutes, using a mixer at high speed until the mixture thickens and fluffy. 10 drops of bovine or pork gelatin was then added to the mixture before shaking the mixture again for 2 minutes. After the mixture was expanded, it was then poured into a container and frozen for 3-5 hours.

2.3.2. Sample measurement. The ice cream sample was extracted using 1 M HCl solution in the ratio of 1:3 (ice cream: 1 M HCl solution). After extraction process, the filtrate obtained was neutralized with demineralized water. The blank used was 300 mL of demineralized water. During the measurement, 100 -500 ppm sample solutions were used under alkaline conditions (pH 9, adjusted using NaOH solution) with QCM system (QCM 200, SRS, USA) as explained in a previous work [17]. The responses of the modified gold QCM sensor toward samples solution were monitored by immersing the QCM sensor into the sample solution during stirring. The modified QCM sensor was also tested to the homemade ice cream samples that have been pretreated as described above.

3. Results and discussion
3.1. QCM sensor modification
The electropolymerization was performed using cyclic voltammetry in 0.1 M aniline at pH 1.5. It showed that the voltammogram peak was gradually increased for further cycles. This reflected that polyaniline were successfully attached to the surface of QCM gold layer sensor by layer along with the cycles. The electropolymerization of aniline was carried out at pH 1.5 because we would like to produce a conductive polymer. This conductive polymer can be obtained if the polymer is formed through head to tail coupling which can only be conducted under acidic pH conditions. While at higher pH (alkaline conditions), the deposited film consist of low chain oligomeric material [22].

3.2. Identification of gelatin in homemade ice cream samples

![Figure 1](image-url) Frequency shift of homemade ice cream samples: pork gelatin (A) and bovine gelatin (B) in the PANI / NiO modified nanoparticle QCM sensor test (black:blanko, red:100ppm, blue:200ppm, pink:300ppm, green:400 ppm and purple: 500ppm).
For further identification, the gold QCM sensor is modified by the compound polyaniline / nickel to identify non-halal gelatin (pork) in homemade ice cream. The purpose of this experiment was to find out the performance of gold QCM sensors modified by polyaniline/nickel compounds in complex matrices (i.e. milk, coconut milk, flavor and coloring). The test results are shown in Figure 1.a-1.b. The results of selective QCM sensor testing of pork gelatin on homemade ice cream samples with the addition of pig gelatin showed an increased frequency response to the ice cream sample (Figure 2.a). On the other hand, test results on homemade ice cream samples with the addition of cow gelatin showed a decreased frequency response to the sample (Figure 2.b). The value of the frequency shift is greater along with the increasing concentration of ice cream added at the time of testing.

The bovine gelatin solution shows a negative frequency shift while the porcine gelatin shows a positive frequency. This different response could be explained through Sauerbrey’s equation (1) below:

\[ \Delta F = \Delta m \times C \]

Where \( \Delta F \) represents the frequency shifts of the QCM sensor, \( C \) is the sensitivity factor of the crystal used (it is 56.6 Hz \( \mu \)g-1 cm2 for 5 MHz AT-cut quartz crystal at room temperature), and \( \Delta m \) is the mass variation per unit area (g cm\(^{-2}\)). When the analyte is attached to the surface of QCM sensor, the mass on the microbalance will increase so that the frequency will decrease (negative frequency shift) [17]. Meanwhile, the positive frequency shift means that the interaction between the sensitive layer and the analyte is relatively strong. The analyte takes off the sensitive layer from the surface of polyaniline/nickel compound modified gold QCM sensor into the solution. The free mobility of the analyte over the sensor layer results positive frequency shift called the anti-Sauerbrey behavior [23].

4. Conclusion
The identification of halal gelatin (bovine) and non-halal gelatin (pork) using gold QCM sensors modified with polyaniline/nickel compounds has been studied. The results showed that bovine gelatin and pork gelatin can be distinguished by the frequency shift response. Bovine gelatin showed a negative frequency shift response whereas a frequency shift response was positive for pork gelatin. The value of the frequency shift increases with increasing concentration of the analyte. The performance of the modified polyaniline/nickel gold QCM sensor was also resulted in no significant interference from the commercial matrix of homemade ice cream samples. This shows that the modified QCM gold sensor of polyaniline/nickel compound has the potential to be applied to distinguish halal (bovine) and non-halal (pork) gelatin.

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