International Journal of Innovative Research and Reviews, 1(1) 1-5

International Journal of Innovative Research and Reviews
ISSN: 2636-8919
Website: www.injirr.com
doi:
Research paper, Short communication, Review, Technical paper

RESEARH ARTICLE

Determination of Hydrogen Peroxide in Foods Using Enzyme-like Behaviors of Nano Iron-Oxide

Sümeyra GÜNDÜZ, Hayrunnisa NADAROĞLU[1,2], Esra TURGUT[1], Azize ALAYLI GÜNGÖR[1,3*]

[1] *Ataturk University, Faculty of Engineering, Department of Nano-Science and Nano-Engineering, 25240 Erzurum, TURKEY*
[2] *Ataturk University, Erzurum Vocational Training School, Department of Food Technology, 25240 Erzurum, TURKEY*
[3] *Ataturk University, Erzurum Vocational Training School, Department of Chemical Technology, 25240 Erzurum, TURKEY*

**Corresponding author E-mail: aalayli@atauni.edu.tr*

HIGHLIGHTS

- > Fe_3O_4 nanoparticles were successfully prepared using green synthesis method.
- > Highly rapid, selective and sensitive detection of H_2O_2 using Fe_3O_4 nanoparticles in some food samples was demonstrated.
- > It seemed advisable to implement this process as a biosensor to determination of H_2O_2 amount.
- > Satisfactory recovery values were obtained for food samples' analysis.

ARTICLE INFO

Received : 10.06.2017
Accepted : 12.05.2017
Published : 12.15.2017

Keywords:
Hydrogen peroxide (H_2O_2)
Green synthesis
Nano-iron-oxide (Fe_3O_4)

ABSTRACT

Hydrogen peroxide (H_2O_2) is a chemical compound that is introduced into food for the food production, processing, packaging and preservation. In many countries it is known that participation in gardens is prohibited and causes food production costs to be lowered even though it is restricted, so it can be informally involved in food and it is known that microorganisms breeding in food and causing deterioration cause increase in H_2O_2 in food. It has been reported that a considerable decrease in the quality of H_2O_2 food, which is present in high quantities in potatoes, leads to loss of vitamins and especially poisoning and cancer. For this reason, it is very important to determine the peroxide level in ppm scale in foods at the residual level. In this study; we aimed to determine hydrogen peroxide using nano-iron-oxide (Fe_3O_4) synthesized by green synthesis method. For this purpose, we determined that hydrogen peroxide determination at 0.15-12.5 µm level can be done linearly by using the reaction that nano-iron-oxide (Fe_3O_4) shows peroxidase enzyme like properties.

Contents

Cite this article Gündüz S, Nadaroğlu H, Turgut E, Alayli Güngör A. Determination of Hydrogen Peroxide in Foods Using Enzyme-like Behaviors of Nano Iron-Oxide. *International Journal of Innovative Research and Reviews (INJIRR)* (2017) **1**(1):1-5

Link to this article: http://www.injirr.com/article/view/6

1. Introduction

Hydrogen peroxide (H_2O_2) is a chemical compound commonly used in the food industry. It is used in the sterilization and packaging stages of food production, mixing, transporting and bottling equipment. H_2O_2 medium is added in the sterilization and packaging steps of the food. Residual analysis of H_2O_2 is needed to remove the added H_2O_2 and then to H_2O_2. Milk and dairy products have been widely used in industry due to its H_2O_2 bactericidal properties.

As a result of excessive H_2O_2 addition to foods or due to accumulation thereof, many undesirable effects can occur. At the beginning of these, important vitamins such as folic acid have been degraded. It has also been determined that high amounts of H_2O_2 taken orally may cause severe gastrointestinal problems. $H_2O_2$was detected in dairy products produced in 2007 and 2011, although Brazilian milk was not allowed to legally add H_2O_2 to extend the life of the milk or to reduce the cost of production [1].

Especially since growing infants and children are commonly consuming milk and dairy products, it is very important to be able to determine the peroxide that can be found in these products. In Japan, the addition of H_2O_2 to commonly consumed fish products and to a kind of noodle, has been reported to cause poisoning due to both the two foods. 390 people were taken to the hospital and the symptoms of poisoning caused by throat burning, nausea, and vomiting, abdominal cramps were reported in May 2000. In addition, there has been reported a case of poisoning upon drinking traditional beverage uzo supplemented with H_2O_2, in Japan. It has been reported that the peroxide was added to the uzo for the purpose of eliminating a toxin caused by Bacillus cereus, but it has been reported that the patients referred to the hospital due to gastroenterological problems [2].

Because H_2O_2 is a powerful bleaching agent and an effective bactericide it is commonly added to food. Chemically it can lead to redox reactions. Heat is readily dissociated by some enzymes such as light metals, alkali solution and catalase and peroxidase. However, H_2O_2 has been reported to have an effect, such as inducing duodenal cancer.

Although many foods in Taiwan do not allow H_2O_2 residues, they are allowed to be used as aquatic products during the processing of gland food. The health authority in Japan is well prepared to thoroughly remove H_2O_2 from food before use, which requires thorough washing of the food. According to the food codex in the USA, the hydrogen peroxide in the aseptic packages should not exceed 0.5 ppm. In the case of a food poisoning case made on the spot, it was determined that hydrogen peroxide is in excess of the amount of hydrogen peroxide [2].

In addition, some food grade peroxide is an indicator of microbial growth and / or degradation of foods. It may also indicate that the food is oxidized and degraded. According to TSE standards, oils are used especially as a measure of compliance with standards during the production of oils and yoghurt in foods.

Our aim in this research is to develop a new method for the detection of hydrogen peroxide residues in a linear and precise manner by using enzyme mimic activities of the nano-iron-oxide particles synthesized by the green synthesis method and also to determine the amount of residual hydrogen peroxide in some greenhouse using this method.

2. Material and Method

2.1. Green Synthesis of Fe_3O_4 nanoparticles

5 mL of the supernatant obtained from the spurge plant (euphorbia) was added to 100 mL of 1 mM $FeCl_2$-$FeCl_3$ solutions and incubated in the magnetic stirrer. At the end of this run, it was observed that the reaction mixture was controlled and turned into a deep red-brown color indicating the formation of Fe_3O_4 nanoparticles. Then, the water was removed by means of an evaporator, and the obtained Fe_3O_4 nanoparticles were dried at 65 ° C for 48 hours [3, 4].

2.2. Characterization of Fe_3O_4 nanoparticles

The magnetic nanoparticles obtained by the green synthesis method were scanned at a wavelength of 290-500 nm using a UV-VIS-NIR spectrophotometer (Shimadzu UV-3600 Plus). In order to investigate the topographical structure of Fe_3O_4 magnetic nanoparticles, images were taken with SEM (Scanning Electron Microscope) (Zeiss brand). Furthermore, in order to determine the sizes of Fe_3O_4 magnetic nanoparticles, XRD (Panalytical Empyrean brand) device and FT-IR (Fourier Transform Infrared) spectrophotometer were used to analyze the bonds formed with the elements [3].

2.3. Investigation of peroxidase-like activity of Fe_3O_4 magnetic nanoparticles

To investigate the peroxidase-like activity of Fe_3O_4 magnetic nanoparticles prepared by green synthesis, the catalytic oxidation of peroxidase substrate ABTS in the presence of H_2O_2 was tested. For analysis; 24 μL of the ABTS solution prepared in 60 mM was mixed with Fe_3O_4 MNP stock solution for 3 min in the magnetic stirrer, after which 10 μL of homogeneous Fe_3O_4, 24 μL of the H_2O_2 solution prepared as different concentration and 185 μL of the acetate buffer prepared at the pH:4.

The reaction solution homogenized in the magnetic stirrer was allowed to stand for 10 minutes for incubation in a water bath adjusted to 45 °C. The tube removed from the water bath was left in the centrifuge for 1 min. to allow separation of the Fe_3O_4 magnetic nanoparticles from the solution. 100 μL of the reaction solution separated by Fe_3O_4 MNPs was added, 900 μL of purified water was added and mixed, and measurement was carried out in a UV-Vis spectrophotometer (417 nm) [5].

2.4. Optimization studies for the method

Optimal reaction conditions have been studied to determine the best working conditions of glucose biosensors after the peroxidase-like activity of the Fe_3O_4 magnetic nanoparticles obtained by the green synthesis method is finalized at the end of the experiment.

2.5. Preparation of the food samples

Preparation of hydrogen peroxide standard concentrations: Peroxide standards are prepared using pure water at different concentrations of 0,15-12,5 μM using 0.1 mM (stock). The standard chart drawn using these standard solutions is then used to determine the amount of peroxide in the food samples.

Fruit juice, milk and similar samples: In order for the peroxide analysis to be performed, for example, samples must be clear and homogeneous. For this purpose, the fruit juices which are in the cloudy structure have been tried to get a clear image by passing them through filter paper. Peroxide analysis was carried out after the sample was diluted by 1/2.

Samples of carbonated beverages and the like: The sample is mixed for 3 minutes at 35 ° C to separate carbonic acid from the sample. Peroxide analysis was carried out after the sample was diluted by 1/2.

Jam and similar samples: After the sample is homogenized, weigh 5 g and complete with 20 ml of pure water. The solution was homogenized with a heater and then passed through a filter paper. Peroxide analysis was carried out by diluting the sample to 1/4.

Honey samples: After stirring the honey, 20 g was taken with spatula and weighed on the sensitive balance. After being homogenized by stirring with a heater at 60 °C for 15 minutes, it was expected to cool down. 5 g of honey sample is weighed and dissolved in a total of 20 mL of distilled water. Peroxide analysis was carried out by diluting the sample to 1/4.

3. Results and Discussion

3.1. Green synthesis results of Fe_3O_4 nanoparticles

In this study, Fe_3O_4 nanoparticles, which have similar properties of the peroxidase enzyme, were obtained by green synthetic method using the extract of the vernal plant. Fe_3O_4 nanoparticles (10 μg protein/mL) were added to the reaction mixture in the rumen extract at a ratio of 2:1 (Fe^{3+}/Fe^{2+}). The highest peak absorbance value of Fe_3O_4 nanoparticles was read at 342 nm. In the subsequent studies, the reference value for nano-iron-oxide was assumed to be 324. The formation reaction of Fe_3O_4 nanoparticles is shown in Figure 1 below.

$$Fe^{2+} + Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O$$

Figure 1 Production reaction of iron oxide NP by green synthesis method.

When the images of the characteristics of the nanoparticle surface synthesized using FT-IR and Zeiss-branded Scanning Electron Microscope (SEM) were examined, it was determined that the Fe_3O_4 nanoparticles were in the form of nanospheres forming a chain with a diameter of 40-70 nm in contact with each other.

It has been found that Fe_3O_4 magnetic nanoparticles synthesized by the green synthesis method exhibit similar properties of the peroxidase enzyme by mimicking real enzymes in Figure 2. ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid] -diammonium salt) is the substrate of the peroxidase enzyme and has been used in our research for this reason. Instead of the peroxidase enzyme, an iron oxide compound which exhibits its properties has been used.

$$H_2O_2 + ABTS \xrightarrow{\text{Peroxidases}} 2H_2O + \text{oxide ABTS}$$

$$H_2O_2 + ABTS \xrightarrow{\text{Nano } Fe_3O_4} 2H_2O + \text{oxide ABTS}$$

Figure 2 Use of nano-iron-oxide instead of peroxidase enzyme.

Gao et al. (2011) published in the study; the fluorometric method in which Fe_3O_4 magnetic nanoparticles were used as a catalyst for hydrogen peroxide determination was investigated. A new fluorometric method has been developed using the peroxidase-like property of Fe_3O_4 magnetic nanoparticles (MNP) for hydrogen peroxide and glucose detection. Fe_3O_4 was radically cleaved to extinguish the fluorescence more effectively and rapidly in the presence of H_2O_2 as a peroxidase mimetic catalyst of MNPs. Under optimum reaction conditions, the hydrogen peroxides could be determined by linear correlation between H_2O_2 concentration of $1.8x10^{-7}$ to $9x10^{-4}$ mol / L. The proposed method was applied for the determination of hydrogen peroxide in human serum samples and satisfactory results were obtained [6].

In another study; A glucose-oxidase nanocomposite and a peroxidase-like protein-Fe_3O_4 based, efficient colorimetric peroxide biosensor have been investigated. Compared with naked MNPs, peroxidase-like casein-MNPs exhibited good catalytic properties, stability, and dispersion. Incorporated casein incorporated in MNPs has been developed to have affinity for H_2O_2 and TMB, which is evidenced by variations in kinetic parameters. A value as low as 0.2 μM H_2O_2 could be detected with a linear range between 0.5 μM and 200 μM H_2O_2 [7].

Song et al. (2017) investigated the peroxidase mimetic activity of Fe_3O_4 nanoparticles based on magnetic hydrogels for hydrogen peroxide detection. Magnetic hydrogels responding to stimuli are prepared in magnetic surfactants, sodium cholate mixtures. The results have proved that the hydrophobicity of the surfactants and the hydration radius of the anions play a crucial role in the gelation process. The prepared Fe_3O_4 nanoparticles exhibit excellent ferromagnetic properties and high peroxidase-like activity and can be used as biosensors for hydrogen peroxide (H_2O_2) and. Fe_3O_4 nanoparticles prepared in a manner suitable for green synthesis, which can be used as a promising biosensor candidate for glucose detection, have been developed [8].

Zhao et al. (2017) published in the study; A biosensor based on 3D graphene-supported Fe_3O_4 quantum dots was used as a biomimetic enzyme for in situ detection of H_2O_2 released from living cells. Hydrogen peroxide (H_2O_2) released from living cells has become important for studying real-time detection, biological phenomena and diseases related to oxidative stress. However, H_2O_2 has still been found to be a major problem due to selective detection in live cells and accurate in situ monitor, low concentration, wide diffusivity and high reactivity.

Electrochemical measurement results show excellent catalytic properties against H_2O_2 with high selectivity and sensitivity (274.15mA M^{-1} cm^{-2}), low detection limit (~78 nM), fast response (2.8s) and excellent reproducibility of Fe_3O_4 / 3DG NCs activity. This enzyme-free biosensor has been successfully used to detect H_2O_2 released from living cells by controlling cell numbers and stimulating drug doses; this has been shown to be of considerable utility in order to understand the critical pathological process of cancer cells [9].

We have developed a method which is linear between 0,15-12,5 μM, we think that it can be preferred because of its easy application knowledge and low cost of experiment even though it is less sensitive than fluorometric principle. In addition, we have observed that lower ppm peroxide determination can be done spectrophotometrically or even chlorometrically (417 nm) using this method when analyzing residuals at desired ppm level in green.

We measured the absorbance values of hydrogen peroxide at 0.17 mM (stock at different concentrations in order to determine the concentration of H_2O_2 in the optimum conditions (5 mM Fe_3O_4, 0-40 ° C, pH: 4). The absorbance (λ) - H_2O_2 Concentration values are plotted in Figure 3. The absorbance at different concentrations of the substrate in the given operating conditions is given in Figure 3.

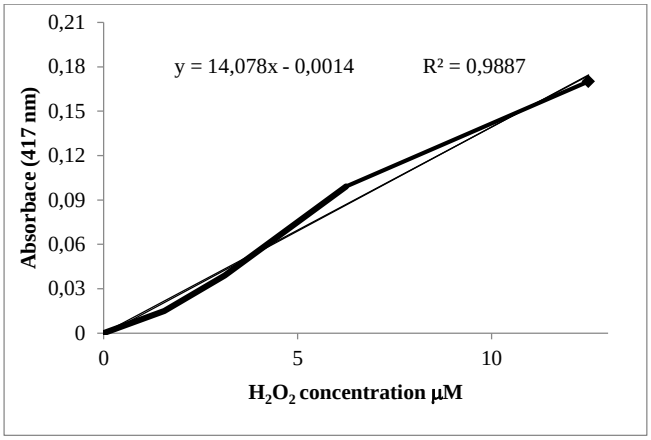

Figure 3 H_2O_2- absorbance (417 nm) standard graphic

It was aimed to make hydrogen peroxide in some foods from the form prepared using the standard graphic obtained.

$$\text{H202 concentration micro M} = \frac{\text{Absorbance}(417\text{ nm}) + 0.0014}{14.078}$$

Table 1 Amounts of hydrogen peroxide in some foods and reference values.

Food Sample	Fe_3O_4 magnetic nanoparticle spectrophotometric measurement method (μM)
Apricot Juice	4.54 μM
Honey	18.38 μM
Milk	3.01 μM
Strawberry Jam	14.97 μM

Hydrogen peroxide is limited due to health hazards that are allowed to be used directly in the food industry in food. Raw poultry to be processed for the purpose of microbial removal can be added at a rate of 0.1% in hot climates where cold storage and transport possibilities are not available. At the same time, 0.4% H_2O_2 is used to prepare the modified water. Wine is used as an antioxidant in the production of egg syrup and in corn syrup production, instant tea production, and as a bleaching agent in the production of cheeses for colored cheeses. In these cases, residual hydrogen peroxide needs to be removed and for this purpose, the average catalase is added [10]. The main antibacterial activity of honey is reported to be hydrogen peroxide from glucose oxidase produced in hypo-pharyngeal glands. In addition, polenta-derived catalase determines the level of hydrogen peroxide in the honey along with glucose oxidase. The antimicrobial effect of honeys varies depending on the variability of plant resources [11, 12].

We have also seen that the H_2O_2 ratio found in our analysis is far below what can be added. We believe that the method can be used more simply and inexpensively without being highly dependent on environmental conditions and without being influenced by proteases.

4. Conclusions

Although the enzymes are excellent catalysts, they have the advantages of disadvantages such as being very easily affected by environmental conditions. Catalytic enzymes lose their activity in a very short time when interacting with protease enzymes present in heat, light, inhibitors and the environment. For these reasons, alternate enzyme-like activity constructs have gained importance nowadays and have been seen as candidates for retaining enzymes. In our work, we have seen that the nano-Fe_3O_4 synthesized by the green synthesis method exhibits a peroxidase-like property due to the redox potential it possesses and that many areas can be used instead of peroxidase enzymes. We have demonstrated one of the usage availability of an enzyme-like structure by identifying hydrogen peroxide in food.

References

[1] Silva RAB, Montes RHO, Richter EM, Munoz RAA. Rapid and selective determination of hydrogen peroxide residues in milk by batch injection analysis with amperometric detection. *Food Chemistry* (2012) **133**:200–4. doi:10.1016/j.foodchem.2012.01.003.

[2] Tanaka A, Iijima M, Kikuchi Y. Determination of hydrogen peroxide in fish products and noodles (Japanese) by gas-liquid chromatography with electron-capture detection. *Journal of Agricultural and Food Chemistry* (1990) **38**:2154–9. doi:10.1021/jf00102a011.

[3] Güngör AA, Gündüz S, Nadaroğlu H, Altundaş A, editors. *Glukoz Tayini İçin Enzim Benzeri Metal Nanopartiküllerin Kullanımının Araştırılması [Investigation of the Use of Enzyme-Like Metal Nanoparticles for Glucose Detection]* (2017).

[4] Karaduman I, Güngör AA, Nadaroğlu H, Altundaş A, Acar S. Green synthesis of γ-Fe_2O_3 nanoparticles for methane gas sensing. *Journal of Materials Science: Materials in Electronics* (2017) **28**:16094–105. doi:10.1007/s10854-017-7510-5.

[5] Güngör AA. Use of Different Plant Derived Peroxidases for the Removal of Phenol from Water. *Asian Journal of Chemistry (ISI),* (2011) **23**:3710-3712.

[6] Gao Y, Wang G, Huang H, Hu J, Shah SM, Su X. Fluorometric method for the determination of hydrogen peroxide and glucose with Fe3O4 as catalyst. *Talanta* (2011) **85**:1075–80. doi:10.1016/j.talanta.2011.05.021.

[7] Liu J, Lu L, Li A, Tang J, Wang S, Xu S, et al. Simultaneous detection of hydrogen peroxide and glucose in human serum with upconversion luminescence. *Biosensors & Bioelectronics* (2015) **68**:204–9. doi:10.1016/j.bios.2014.12.053.

[8] Song S, Liu Y, Song A, Zhao Z., Lu H, Hao J. Peroxidase mimetic activity of Fe3O4 nanoparticle prepared based on magnetic hydrogels for hydrogen peroxide and glucose detection. *Journal of Colloid and Interface Science* (2017) **506**:46–57.

[9] Zhao, Y., Huo D., Bao J., Yang M., Chen M., Hou J., Fa H. and Hou C. Biosensor based on 3D graphene-supported Fe_3O_4 quantum dots as biomimetic enzyme for in situ detection of H_2O_2 released from living cells. *Sensors and Actuators B: Chemical* (2017) **244**:1037–44.

[10] Bağcı U, Temiz A. Taze Sıkılmış Meyve Sularının Mikrobiyolojik Kalitesi [Microbiological Quality of Fresh Fruit Juices]. *Orlab Online Mikrobiyoloji Dergisi [Orlab Online Journal of Microbiology]* (2006) **4**:1–20.

[11] White JW, Subers MH, Schepartz AI. The identification of inhibine the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochimica et Biophysica Acta (BBA)* (1963):57–70.

[12] Weston R. Identification and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chemistry* (2000) **70**:427–35. doi:10.1016/S0308-8146(00)00127-8.