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ORIGINAL ARTICLE

Green synthesis of *Ananas comosus* (L.) Merr. based-silver nanoparticles and determination of their potential in electrochemical detection of H_2O_2

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Abstract
It is possible to synthesize silver nanoparticles, which are used to increase the conductivity of carbon paste electrodes used in the design of biosensors that enable the determination of the amount of H_2O_2 in foods quickly, practically, and at low concentrations. Silver nanoparticles used in electrode modification could be obtained cheaply and eco-friendly by green synthesis technique in which bio-waste is used as electron precursor. In this study, the bio-waste leaves of pineapple (*Ananas comosus* (L.) Merr.) were used in the green synthesis of silver nanoparticles (A-AgNPs). Main aim of this study is to determine the potential usage of synthesized A-AgNPs in the area of new biosensor applications. It was determined that A-AgNPs had maximum absorbance at ~ 450 nm, sized between ~ 40 and 70 nm, strong signal at 3 keV, and silver peaks representing 111°, 200°, 220°, and 311° at 2θ. The carbon paste electrode (CPE) was modified with the characterized A-AgNPs and linear detection range of H_2O_2 was determined as 0.1–1000 µM. It was determined that silver nanoparticles obtained from waste pineapple leaves increased the surface functionality and enabled the determination of H_2O_2 in a wider concentration range. It could be concluded that the synthesized nanoparticles have the potential to be used in biosensor technology. Therefore, bio-wastes can be used for nanoparticle synthesis in an environmentally friendly and economically feasible way.

Keywords Biosensors · Nanoparticles · Food waste · Biological synthesis · Food quality · Sustainability

Highlights
- H_2O_2 is a toxic chemical and should be determined even low concentrations quickly.
- Biosensors provides to determine H_2O_2 by modified carbon paste electrodes (MCPE).
- MCPE could be prepared by green synthesized silver nanoparticles (AgNPs).
- AgNPs could be obtained by recovering waste food bio-components in green synthesis.
- The eco-friendly AgNPs could be used to determine toxic H_2O_2 by biosensors.

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1 Introduction

Hydrogen peroxide (H_2O_2) is a colorless, odorless, and unstable substance that could be degraded by various factors and converted into water and oxygen [1]. In biochemical processes, H_2O_2 could be formed as a result of enzymatic or non-enzymatic reactions [2]. H_2O_2 acts as a precursor of hydroxyl radical in the presence of metal ions such as iron and copper with its oxidizing property. In particular, it reacts with iron in proteins to form highly oxidized reactive iron forms. This could cause the initiation of radical reactions that cause protein-lipid peroxidation in the cell membrane and disruption of the DNA chain. H_2O_2 is a product formed in many reactions catalyzed by some oxidase enzymes [3]. Longer exposure to H_2O_2 damages the skin and organs due to its strong oxidizing ability. In addition, according to the American Food and Agriculture Organization, the H_2O_2 content of foods should be at a certain level. In case of milk, it should not be more than 0.05% and should be monitored attentively [4]. H_2O_2 could be analyzed by using traditional titration method which have a long process time,

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chromatography, fluorosensitive, chemiluminisensitive, spectroscopic, and electrochemical methods [4]. Methods such as titration and chromatography have many disadvantages due to the time of process and toxic chemicals usage during processing. In contrast to these traditional analysis methods, there is a need for a practical, quicker, toxic chemical-free, and cost-effective method for the analysis of H_2O_2. Biosensors allow the determination of formed directly [5] or enzymatically [6]. Biosensors have the determination ability of H_2O_2 quickly, cost effectively, practically, and even at low concentrations.

It is important for a sustainable future, household wastes, which increase with population growth, should be recycled due to the components such as proteins, phenolics, flavanoids, terpenes, and alkaloids. Especially, phenolic compounds of household wastes have bioactive-protective properties and antioxidant, anticancer, antidiabetic, and antiallergic properties [7]. The lack of recovery of these components, which have a wide range of both direct and indirect uses, constitutes a great loss for both the environment and the economy. Using of household fruit and vegetable wastes in the branch of technologywill contribute to sustainability. The use of these components in nanoparticle synthesis within the context of nanotechnology is a good opportunity and alternative for recovery in this field.

Nanotechnology is a branch of science that aims to obtain nano-sized materials and offers innovations in many applications with nanoparticles [8]. Nanoparticles have many advantages with their different properties such as their small size, shape, chemical composition, and purity [9]. Materials using nanoparticles show higher reactivity and mechanical resistance, better electrical, and thermal properties. They find applications in many fields, especially in biomedicine. They are used for various applications such as chemical sensors, antimicrobial activity, medical imaging, cancer diagnosis and treatment, cosmetic applications, specific drug release, and wound healing [10].

Physical, chemical, or biological methods are used for the synthesis of nanoparticles [11]. Physical and chemical methods have many disadvantages due to their high cost and toxic chemicals [12]. Environmentally, friendly green (biological) synthesis method, an alternative to these methods, which supports recovery, provides the opportunity to work with less hazardous reagents in terms of environment-health [13]. This method also has some advantages like minimizing the formation of by-products, having high efficiency, being easily applicable, and low-energy requirement. Therefore interest for this method has increased significantly in recent years [14].

In the green synthesis method, plants, bacteria, algae, and fungi could be a good electron precursor for the synthesis proces. Parts of plants such as roots, stems, leaves, flowers, fruits, and seeds are widely used in nanoparticle synthesis due to their reducing agents [15]. The bioactive components contained in household waste are evaluated and could be a source of reducing agents in green synthesis [9]. Nanoparticles such as aluminum, gold, copper, zinc, iron, silver, cadmium, cobalt, and lead are among the well-known metallic nanoparticles in the literature, preferred and popular in scientific studies. Metallic nanoparticles are preferred in many branches due to their physicochemical properties, high surface area and non-toxicity [16].

Silver nanoparticles (AgNPs) are used in many different fields, including antimicrobial activity [17], bactericidal activity [18], wound healing [19], personal hygiene [20], health care [21], anticancer activity [22] skin diseases [23], storage and preservation [24], environment [25], food packaging [26], and cosmetics [27] (Table 1). In addition, the use of AgNPs in the fields of sensor spectroscopy by increasing conductivity and in the pharmaceutical industry as an alternative to antibiotics with its antibacterial properties is becoming widespread [28].

Pineapple (*Ananas comosus* (L.) Merr.) is a tropical plant with edible fruit from the pineapple family (*Bromeliaceae*) that grows in warm countries. The fruit is large, with a bunch of leaves resembling a crown. It is one of the world's leading marketable fruit crops, grown in many tropical and subtropical countries. Pineapple (*Ananas comosus* (L.) Merr.) has many nutrients and bioactive compounds such as protein, minerals, lipids, vitamin C, phenolic compounds, flavonoids, and carotenoids contained in its fruit, peel, and leaves. For this reason, it is even used as medicine in some countries [31]. The waste parts of pineapple have the potential as electron donors with its bioactive components in using the green synthesis of nanoparticles [32] (Fig. 1).

AgNPs synthesized by green synthesis method could be used in the modification of carbon paste electrodes (CPE) as working electrodes in biosensor design [9]. The usage of nanoparticle-modified carbon paste electrodes (MCPEs) has greatly increased in recent years due to their many advantages such as easy and quick preparation, low-cost, non-time-consuming surface regeneration, ability to contain many materials at the same time, wide potential operating range, and low background currents [33]. For this purpose, A-AgNPs were synthesized from pineapple leaves by green synthesis to obtain a sustainability and environment-health friendly electrode. The CPE was modified with A-AgNPs and the potential of the modified electrode for electrochemical determination of H_2O_2 was determined. It was found that the modification increased the surface area and conductivity where the reaction would take place, so that higher response currents was obtained. With the electrochemical properties of A-AgNPs practical, low-cost, and environmentally friendly A-AgNPs-MCPE was obtained

Table 1 Synthesis of AgNPs by using different sources

Plant or biological source	Reaction conditions	Average size (nm)	Shape	Biomedical application	Ref
Tea leaves (*Camellia sinensis* L.)	40 °C; 40 min	59–93	Spherical	Amperometric biosensor to determine glucose levels	[9]
Chestnut (*Castanea sativa*) honey	30, 60, and 90 °C; ~ 2 h at room temperature	27–55	Spherical	Antioxidant, antibacterial, and enzyme inhibition effects	[15]
Padina gymnospora (brown algae)	Room temperature for 10 min	5–50	Truncated octahedral	Bactericidal activity against *Escherichia coli*, *Lactococcus lactis*, and *Klebsiella pneumoniae*	[18]
Nigella sativa (black cumin, seeds)	75 °C; 2 days	3.47	Spherical	Anticancer activity against human breast (MDA-MB-231) and cervical	[22]
Prunus × *yedoensis* (gum extract)	pH 8; gum extract concentrations of 7% and 8% 30 min	10–50	Spherical	Antifungal against *Colletotrichum acutatum* and *Cladosporium fulvum*	[29]
Punica granatum (pomegranate, crusts)	Room temperature Ultrasonication 24 h	20.12	Spherical and cubes	Antiproliferation effect and enhanced apoptosis against human breast adenocarcinoma cell line (MCF-7)	[30]
Waste pineapple leaves	60 °C; 60 min	~ 40–70	Spherical	Determination of H_2O_2 released as a product of various enzymatic reactions	***This study***

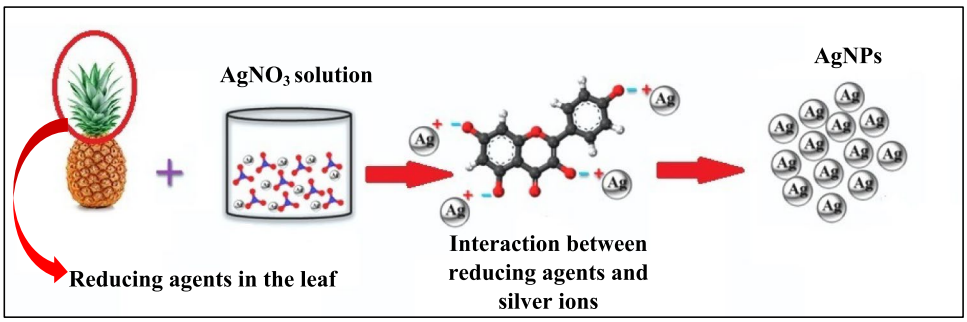


Fig. 1 Schema of the synthesis of AgNPs [7]

for the determination of H_2O_2. In literature, it was seen that silver nanoparticles were obtained using different parts of pineapple, fruit part [34], leaf [35], outer peel [36], and fruit peels [37], but these nanoparticles were not used in the determination of H_2O_2. The potential of the using A-AgNPs in biosensor designing was determined. Thus, the recovery of domestic wastes and the potential for use in the field of nanotechnology were revealed. In these aspects, the findings are novel and the study is innovative.

2 Material and methods

Pineapple leaves, separated as waste, were obtained from a local market. Chemicals used throughout the study were of analytical purity and were obtained from Sigma-Aldrich.

Electrochemical analyses were performed using a 1230-A model device from CHI. All amperometric measurements during the experiments were performed in a three-electrode electrochemical cell. The working electrode was a 0.3-cm diameter carbon paste electrode (teflon material and specially prepared), the reference electrode was Ag/AgCI (BAS RE-5B), and the counter electrode was a platinum wire electrode (MW-1032). The pH was measured with a HANNA HI-8424 pH meter. Weighing was made using a Radwag WAA 200/C/2 balance. DLAB D1524R high-speed refrigerated model was used for centrifugation. Hach DR/4000U spectrophotometer, ZEISS/Supra 40 VP electron microscope model, Thermo Fisher spectroscopy device, and GNR APD 2000 PRO X-ray diffractometer were used for the characterization of nanoparticles.

2.1 Synthesis and characterization of silver nanoparticles

Green leaves of pineapple were washed and dried in an incubator at 50 °C. The dried leaves were cut into small pieces with a blender. Fifteen grams of dry leaves were added to a beaker and 300 mL of distilled water was added to it. The extraction process was carried out with maceration in a magnetic stirrer at 30–40 °C for 1 h. After 1 h, the extract was cooled to room temperature. Then, the extract was filtered with Whatman No. 1 filter paper and the filtered extract was kept for further use [38].

The obtained extract was mixed with 0.05 M $AgNO_3$ solution at a ratio of 1:1 (v/v). The formation of nanoparticles was observed with a darkening of color at a constant speed with a magnetic stirrer at 60 °C for 1 h (Fig. 2) [38]. The prepared solution was centrifuged at 9000 rpm for 10 min to obtain pineapple leaves-based silver nanoparticles (A-AgNPs).

Ultraviolet visible (UV–Vis) spectrophotometry, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction analysis (XRD) methods were used to characterize the synthesized nanoparticles.

2.2 Electrochemical measurements: preparation of carbon paste electrode (CPE) and modified carbon paste electrode (MCPE)

A triple electrode system was preferred in the experimental studies. These electrodes were a reference electrode Ag/AgCl (3 M KCl)—counter electrode platinum wire—working electrode carbon paste electrode. The carbon paste electrode was modified with obtained A-AgNPs to use in electro-oxidation studies. Electrochemical measurements were analyzed using the measuring current against time, amperometric *i-t* method.

To prepare carbon paste electrode (CPE), 65 mg graphite powder was weighed with a sensitive balance, 30 µl mineral oil (nujol) was taken with a micropipette, and added to the graphite powder. These two substances were mixed homogeneously. The electrode chamber was filled without any empty space. The surface of the CPE was cleaned and polished with a special pad and washed with distilled water [2].

For the modified carbon paste electrode (MCPE), A-AgNP was added to the prepared mixture (graphite powder + nujol). Two identical MCPEs were prepared with different amounts of A-AgNP (10 mg and 15 mg) [9].

To determine the sensitivity of CPE and MCPE, the anodic currents formed by the oxidation of H_2O_2 were compared. For this purpose, 9 mL of 0.1 M pH 7.0 phosphate buffer (NaH_2PO_4-$Na_2HPO_4 0.2H_2O$) and 1 mL 1 M NaCl solution were added to the working cell. The potential was set to + 0.7 V, at which the H_2O_2 oxidation. CPE and A-AgNPs-MCPE were equilibrated at + 0.7 V. After the equilibrium current of the system was determined, increasing concentrations of 1.0×10^{-7}–1.0×10^{-3} M H_2O_2 solutions were added to the solution mixture. After each concentration addition, the working cell was mixed with a magnetic stirrer for 1 min and the response current was recorded at the end of the 200-s measurement period. The response currents

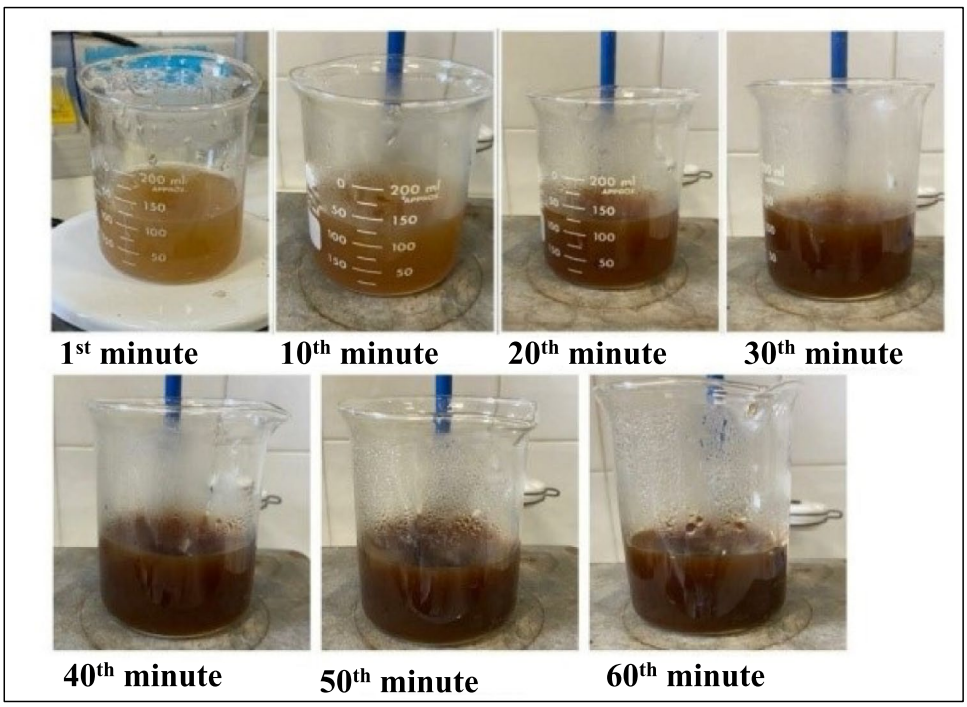


Fig. 2 Formation of AgNPs

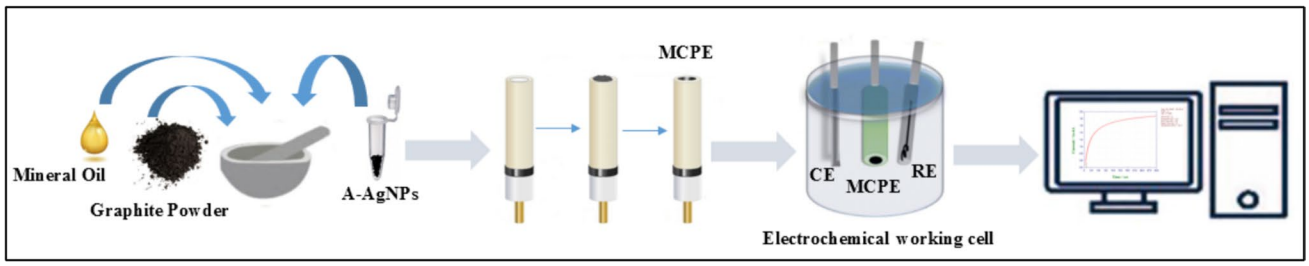


Fig. 3 MCPE preparation and working cell schematic [9]

were recorded and subtracted from the equilibrium current to determine the Δi current differences—graphed and the results were discussed (Fig. 3).

3 Results and discussion

3.1 Ultraviolet visible (UV–Vis) spectroscopy

The formation of the synthesized AgNPs was confirmed by color change and spectroscopic analysis. It was observed that the color of the solution changed from yellowish orange to dark brown at the end of the reaction (Fig. 2). This color change is a strong sign of AgNP formation and indicates the excitation of the surface plasmon in the silver-associated nanoparticle. This supports the transformation of silver in ionic form (Ag^+) to metallic silver (Ag^0). AgNPs give a specific absorbance in the range of 400–450 nm in scans performed between 200 and 800 nm for the detection of AgNPs [39]. UV–Vis absorption spectra of AgNPs were taken in the range of 200 and 800 nm. When the absorption spectrum given in Fig. 4b is examined, it was seen that AgNPs have maximum absorption at ~ 450 nm. As expected, the surface plasmon absorption of silver-based nanoparticles was found to be at maximum peak intensity at ~ 450 nm as expected. Studies in the literature also showed that the characteristic band in the UV absorption spectra of some synthesized AgNPs was 417 nm [40], ~ 430 nm [41], 436 nm [42], 450 nm [43], ~ 450 nm [44], and 461.25 nm [45].

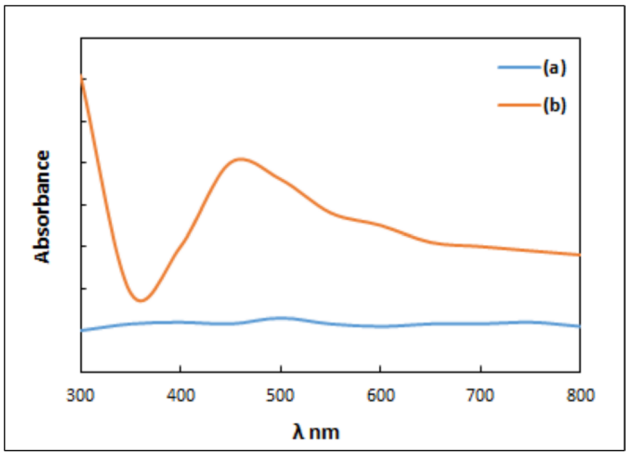


Fig. 4 UV–Vis spectra of (a) waste pineapple leaves extract and (b) solution containing A-AgNPs

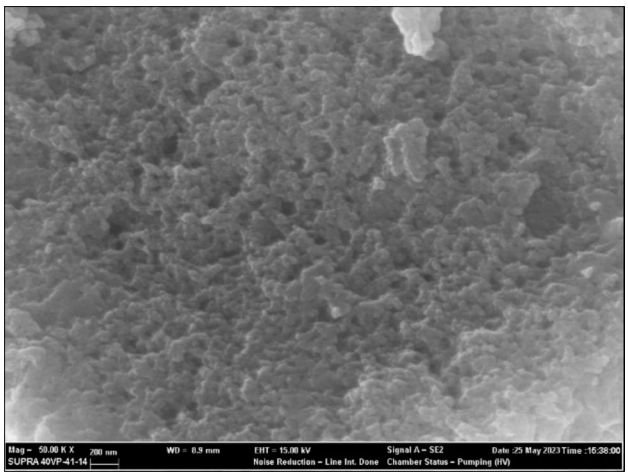


Fig. 5 SEM analysis of A-AgNPs

3.2 Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX)

The morphology and size details of AgNPs were determined by SEM analysis (Fig. 5). Generally, spherical shaped nanoparticles were observed. Spherical nanoparticles are generally more effective than rods or cubes due to a higher surface area, enhanced interaction and better penetration [46]. As a result of SEM analysis, the size of A-AgNPs was observed between ~ 40 and 70 nm in the analyzed area. Nanoparticle sizes in other studies where AgNP synthesis were determined as 50–90 nm [47], 59–93 nm [11], 27–55 nm [17], and 10–50 nm [29].

The energy-dispersive X-ray spectroscopy (EDX) image of the A-AgNPs is given in Fig. 6. In the EDX analysis for AgNPs, the characteristic peaks in the spectrum for silver are

observed around 3 keV due to surface plasmon resonances with weak carbon, oxygen, and nitrogen peaks. The analysis for this study also showed a strong signal peak at 3 keV for A-AgNPs. This value was compatible with other studies [38]. It was thought that weak signals such as oxygen and carbon seen in the EDX profile could be due to biomolecules on the surface of the nanoparticles [48].

3.3 Fourier transform infrared spectroscopy (FTIR)

FTIR also allows the verification of investigations such as the incorporation of functional molecules into carbon nanotubes, graphene, and silver-gold nanoparticles or the detection of enzyme–substrate interactions [49]. Functional groups involved in a plant-derived reduction could be identified using the FTIR spectroscopy. It was observed that the main peaks involved in reduction belong to – OH, C = O, and – C–C bonds [9]. The shifts in the peaks in the spectra between the extract and filtrate extract indicate that – OH, – CN, and C = O groups were involved in the reduction [50]. The band observed at 3320.77 cm^{-1} in pineapple leaves extract shifts to 3315.66 cm^{-1} in the filtrate extract after synthesis. This slightly flat band indicates the presence of intermolecular hydrogen bonding between – OH groups in alcohols and phenols originating from the plant extract used in the synthesis. The band shifting from 1633.16 cm^{-1} in the extract to 1634.14 cm^{-1} in the filtrate extract was due to the stretching modes of the – CO carbonyl group due to – NH amide bonding. Phenolic compounds and flavonoids also vibrated strongly at this wavelength. The peak at ~ 600 nm corresponds to C–Cl stretching vibrations of alkyl halides (Fig. 7).

These results obtained and discussed were compatible with other studies in the literature [51, 52]. The difference in absorption bands between the spectra of pineapple leaves extract and filtrate extract sample causes a shift between ± 1 and 10 cm^{-1}. According to this comparison, it was shown that the synthesis of nanoparticles with plant extracts due to some metabolite functional groups such as amines, alcohols, ketones, aldehydes, and carboxylic acids [53].

3.4 X-ray diffraction (XRD) analysis

The morphology of the crystal structure of A-AgNPs obtained by the green synthesis method was analyzed by XRD (GNR APD 2000 PRO X-ray diffractometer) in the range $15° \leq 2\theta \geq 90°$. In the XRD analysis results, peaks 38.29, 46.17, 64.29, and 77.78 corresponding to 111°, 200°, 220°, and 311° at 2θ, represent the spherical crystal structure of silver (Fig. 8). That these peaks 111°, 200°, 220°, and 311° was belong to AgNPs was supported by other studies in the literature [54, 55]. The crystal size of A-AgNP obtained in this study was calculated as 32.80 nm by the Debye–Scherrer equation, using the highest peak angle of 38.29. AgNPs sizes in other XRD analysis's 23.30 nm [42], 26.08 nm [40], 36.88, and 40.19 nm [41].

(Debye–Scherrer equation: $D = K\lambda/(\beta \cos\theta)$, D = nanoparticle size, K = constant value (0.90), λ = X-ray wavelength value (0.15418 nm), $\beta = \pi/180 \times$ FWHM, FWHM: half of the highest peak value, θ = half of 2θ, the diffraction angle between the incident and diffracted X-ray of the highest peak) [56].

3.5 Determination of H_2O_2 sensitivity of A-AgNPs-MCPE

To compare the sensitivity of carbon paste electrode (CPE) and modified carbon paste electrode (A-AgNPs-MCPE) to

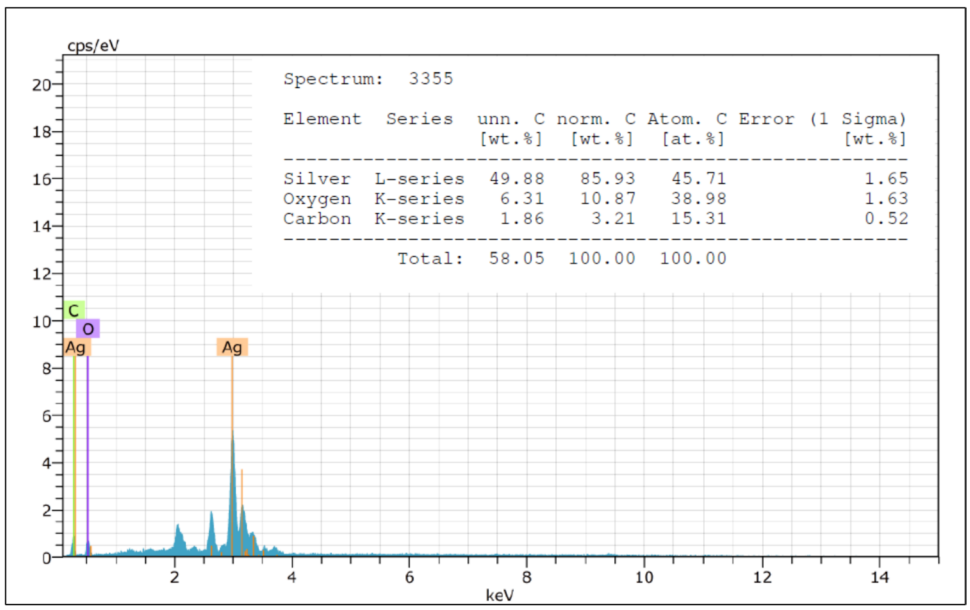


Fig. 6 EDX analysis of A-AgNPs

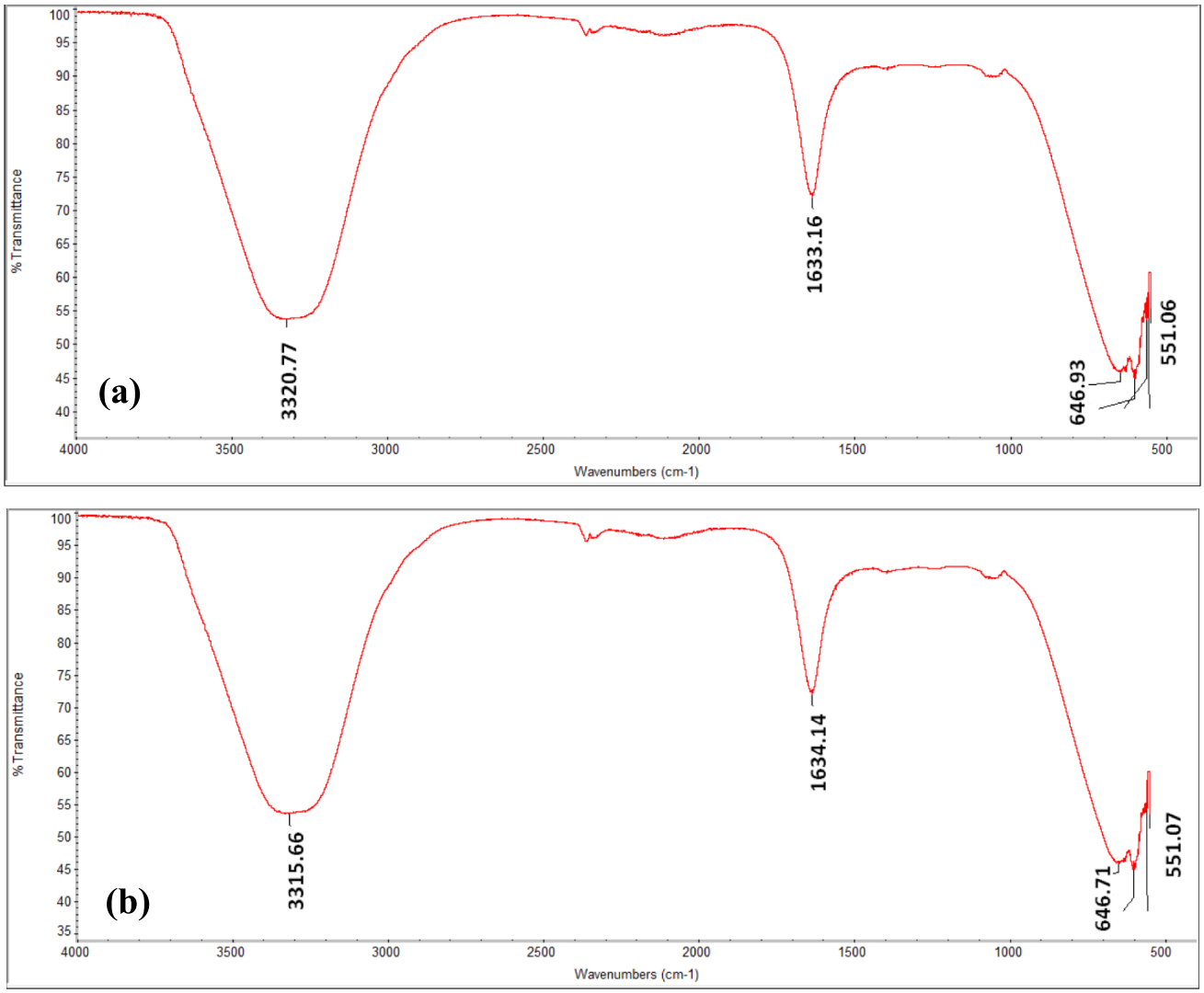


Fig. 7 FTIR analysis (**a**) waste pineapple leaves extract, (**b**) remaining solution after the separation of A-AgNPs filtered after synthesis

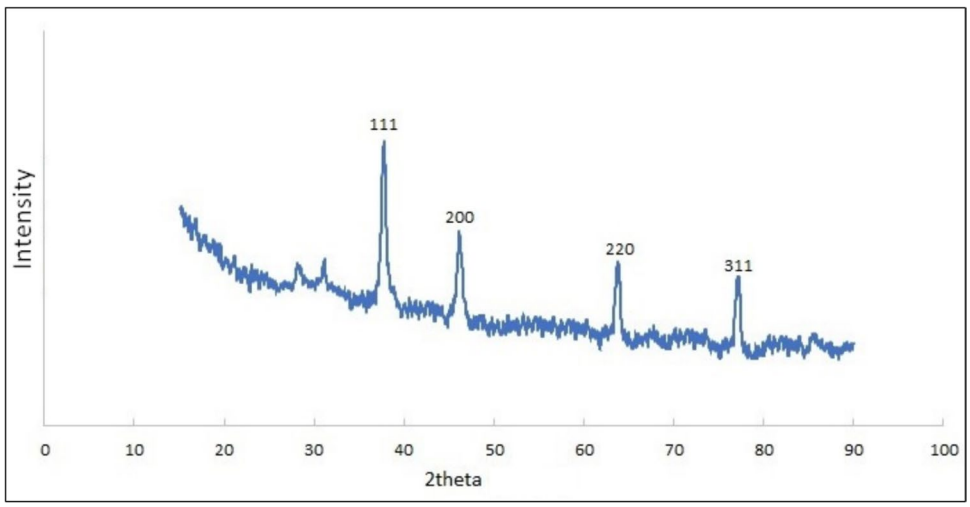


Fig. 8 XRD analysis of A-AgNPs

H_2O_2, the anodic currents generated by H_2O_2 in the working cell were recorded (Fig. 9).

MCPE and CPE were equilibrated at a constant potential of + 0.7 V and then the equilibrium current was recorded [58]. The concentration of H_2O_2 in the working cell was added in the range of 1.0×10^{-7}–1.0×10^{-3} M. The response currents obtained against H_2O_2 concentration were recorded and subtracted from the equilibrium current and the Δi current differences were plotted [59] (Fig. 10). The results obtained for the CPE and MCPE working electrodes prepared to determine the sensitivity to H_2O_2 were compared in Fig. 10 and the optimum amount of A-AgNP was determined. Although the R^2 (determination coefficient) value and linearity were good for both MCPEs, the highest response current was determined with the electrode prepared with 15 mg A-AgNP. When the graph was analyzed, it was seen that the anodic currents of MCPE prepared with 15 mg A-AgNP were ~ 2 times higher than CPE. The linear detection range of the H_2O_2 was determined as 0.1–1000 µM. These ranges in the electrochemical determination studies of H_2O_2 using traditional physical and chemical AgNP synthesis methods were follows as: 0.01–30 µM and 60–600 µM [60]; 6.7–668 µM [61]; 100–1000 µM [62]. As a result of the modification of CPE with A-AgNPs, it was determined that the surface area and electrical conductivity on which the reaction takes place increased, increasing the sensitivity so that high response currents could be obtained even at low H_2O_2 concentrations, and the linear detection range was also determined to be low and in a wide concentration range.

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

Fig. 9 Decomposition reaction of H_2O_2 [57]

4 Conclusion

Recycling of wastes, sustainability, and increasing the initiatives related to recycling issues are very important for the environment and human health. Re-evaluation and recycling of fruit-vegetable wastes, called as household waste due to the bioactive agents in their content, could contribute greatly to the studies to increase these initiatives. On the other hand, determination of H_2O_2 in areas such as health, food, and agriculture with high sensitivity and even at low concentrations and develop alternative methods to traditional methods are important for both economy and a sustainable environment. For this purpose, an extract was prepared using pineapple leaves separated as waste in markets and households, as a reducing agent source. The synthesis of A-AgNPs was obtained by using an environmentally friendly, low-cost, simple, fast, and green synthesis method. The formation of the synthesised A-AgNPs was characterized by UV–Vis spectroscopy, FTIR spectroscopy, EDX and XRD analyses. SEM images showed that the A-AgNPs were in spherical morphology. The characterized (Table 2) A-AgNPs were used for electrode modification and MCPE was prepared. The potential of the prepared electrode to analyze H_2O_2 was determined. Since A-AgNPs increase the surface area and electric current where the reaction will take place, an electrode that enables high response currents even at low analyte concentrations was obtained. The recovery of bioactive components contained in food wastes and determination of their potential use in the determination of food spoilage products such as H_2O_2 is important for the sustainability of both food and technology industries. It could be concluded that by determining the potential usage of the biosensor designed with nanoparticles obtained from biowastes in the determination of H_2O_2, this study has revealed the potential

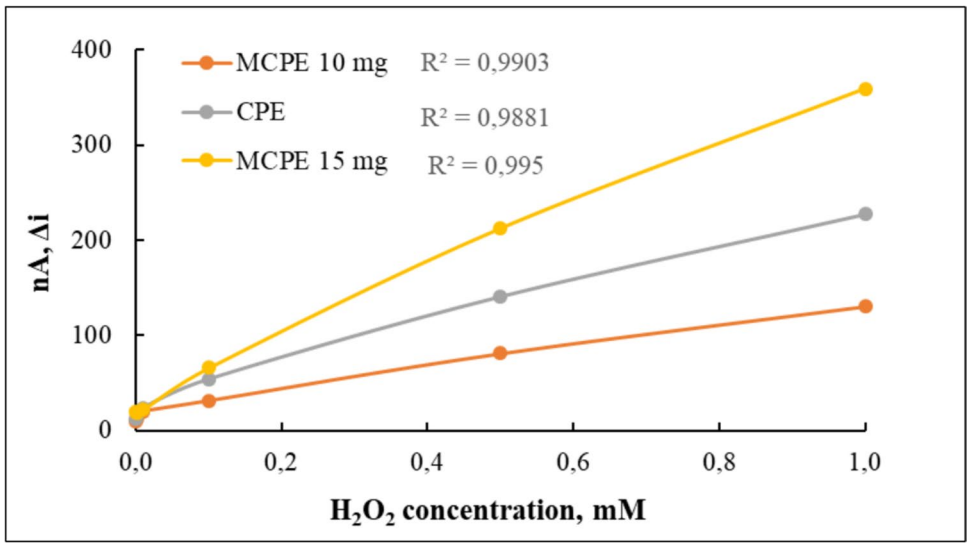


Fig. 10 Determination of H_2O_2 sensitivity of CPE–MCPE

Table 2 Characteristics of the synthesized A-AgNPs

UV–Vis spectroscopy band	SEM analysis size	EDX analysis	XRD analysis
~450 nm	~40–70 nm	3 keV	111°, 200°, 220°, and 311° at 2θ and 32.80 nm

application of household wastes in the field of nanotechnology contrubiting their recycling.

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Author contributions Onur Can Bodur: writing–original draft, formal analysis. Merve Keskin: writing–review and editing, methodology, data curation, conceptualization. Şaban Keskin: review and editing, supervision, project administration. Fatma Arslan: review and editing, supervision, project administration, methodology, data curation, conceptualization.

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Declarations

Competing interests The authors declare no competing interests.

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