Colorimetric Detection Based on Localised Surface Plasmon Resonance Optical Characteristics for the Detection of Hydrogen Peroxide Using Acacia Gum–Stabilised Silver Nanoparticles

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ABSTRACT: The use of nanoparticles in sensing is attracting the interest of many researchers. The aim of this work was to fabricate Acacia gum–stabilised silver nanoparticles (SNPs) using green chemistry to use them as a highly sensitive and cost-effective localised surface plasmon resonance (LSPR) colorimeter sensor for the determination of reactive oxygen species, such as hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). Silver nanoparticles were fabricated by the reduction of an inorganic precursor silver nitrate solution (AgNO\textsubscript{3}) using white sugar as the reducing reagent and Acacia gum as the stabilising reagent and a sonication bath to form uniform silver nanoparticles. The fabricated nanoparticles were characterised by visual observation, ultraviolet-visible (UV-Vis) spectrophotometry, transmission electron microscopy (TEM) analysis, energy-dispersive X-ray spectroscopy (EDAX), thermogravimetric analysis (TGA), and Fourier transform infrared spectroscopy (FT-IR). The TEM micrographs of the synthesised nanoparticles showed the presence of spherical nanoparticles with sizes of approximately 10 nm. The EDAX spectrum result confirmed the presence of silver (58%), carbon (30%), and oxygen (12%). Plasmon colorimetric sensing of H\textsubscript{2}O\textsubscript{2} solution was investigated by introducing H\textsubscript{2}O\textsubscript{2} solution into Acacia gum–capped SNP dispersion, and the change in the LSPR band in the UV-Vis region of spectra was monitored. In this study, it was found that the yellow colour of Acacia gum–stabilised SNPs gradually changed to transparent, and moreover, a remarkable change in the LSPR absorbance strength was observed. The calibration curve was linear over 0.1–0.00001 M H\textsubscript{2}O\textsubscript{2}, with a correlation estimation (R\textsuperscript{2}) of .953. This was due to the aggregation of SNPs following introduction of the H\textsubscript{2}O\textsubscript{2} solution. Furthermore, the fabricated SNPs were successfully used to detect H\textsubscript{2}O\textsubscript{2} solution in a liquid milk sample, thereby demonstrating the ability of the fabricated SNPs to detect H\textsubscript{2}O\textsubscript{2} solution in liquid milk samples. This work showed that Acacia gum–stabilised SNPs may have the potential as a colour indicator in medical and environmental applications.

KEYWORDS: Silver nanoparticles, white sugar, Acacia gum, hydrogen peroxide, localised surface plasmon resonance

Introduction

Noble metal nanomaterials, such as gold and silver, have attracted the attention of researchers because of their distinctive properties, such as large surface energies, unusual optical properties, chemical stability, good conductivity, and catalytic and antibacterial activity.\textsuperscript{1-3} Silver nanoparticles (SNPs) have been used as optical receptors, polarising filters, catalysts, biolabels, and antimicrobial agents.\textsuperscript{4,5} Silver nanoparticles can be monitored using a ultraviolet-visible (UV-Vis) spectrophotometer with specific optical characteristics using localised surface plasmon resonance (LSPR) in the visible range (350-800 nm).\textsuperscript{6-8}

Localised surface plasmon resonance is excited when electromagnetic radiation interacts with nanoparticles to cause excitation of the conduction electrons. Previous works have shown that LSPR was affected by the composition, size, and shape of SNPs.\textsuperscript{9} Localised surface plasmon resonance has been used to monitor biomolecular interactions, for example, enzymatic reactions,\textsuperscript{10} DNA hybridisation,\textsuperscript{11,12} and antigen-antibody reactions.\textsuperscript{13}

Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is formed in living organisms as a by-product of oxygen metabolism, and it plays an important role in oxidative stress as a reactive oxygen species (ROS). Hydrogen peroxide can be formed by enzymatic reaction; hence, this method can be used to measure target molecules, such as glucose, alcohol, and lactates, based on enzymatic reaction.\textsuperscript{15} Previous studies using the LSPR-based optical method with SNPs to monitor target molecules in medical applications are limited in the literature, although there are a few such studies.\textsuperscript{15,16} Filippo et al\textsuperscript{17} monitored LSPR optical characteristics and dispersion condition changes for the determination of H\textsubscript{2}O\textsubscript{2} using polyvinyl alcohol–capped SNPs. In addition, Endo et al\textsuperscript{18} used polyvinylpyrrolidone-stabilised SNPs as an LSPR colorimetric sensor for monitoring H\textsubscript{2}O\textsubscript{2}. Tashkhouirian et al\textsuperscript{19} determined H\textsubscript{2}O\textsubscript{2} using LSPR optical characteristics with SNPs. A recent experimental study from Shrivast et al\textsuperscript{16} fabricated SNPs capped with tartaric acid to use them as an LSPR sensor for monitoring chromium.

Silver nanoparticles have been synthesised using chemical stabilising reagents, such as sodium citrate, ascorbate, and sodium borohydride,\textsuperscript{20-22} by photo-reduction in reverse micelles\textsuperscript{23} and by radiation–chemical reduction.\textsuperscript{24} Many of

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these methods are of high cost and need to use temperature, pressure, energy, or toxic chemicals, and they are not easily scaled up for the large-scale fabrication of nanoparticles. In addition, toxic chemicals used could be absorbed on the surface of the SNPs, thus limiting their application. Other methods that can be used for the fabrication of SNPs are biological methods based on using microorganisms, enzymes, or fungus; however, these methods need special culture preparation and isolation methods for the fabrication of SNPs. A green chemistry route for the fabrication of SNPs would have many advantages compared with other methods, such as facile fabrication, environmentally friendly, elimination of the need for any special culture preparation and isolation method, low cost, low yields, easy scaling up for the large-scale fabrication of nanoparticles, and not consuming a lot of energy.

To the best of our knowledge, there are only a limited number of papers in the literature on using SNPs as a colorimeter sensor for the determination of $\text{H}_2\text{O}_2$; therefore, the purpose of this research was to detect $\text{H}_2\text{O}_2$ solution based on LSPR using Acacia gum–stabilised SNPs as a model case. Silver nanoparticles were fabricated using an eco-friendly method to avoid using toxic or hazardous chemicals. The fabrication was performed using white sugar as a reducing reagent for the silver nitrate salt ($\text{AgNO}_3$) and Acacia gum as a stabilising reagent to fabricate SNPs under ultrasound irradiation and with alkali-ration of the reaction by adding sodium hydroxide (NaOH). The prepared materials were characterised using transmission electron microscopy (TEM), energy-dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FT-IR), and thermogravimetric analysis (TGA). The LSPR optical characteristics of the SNPs, thus limiting their application. Other methods that can be used for the fabrication of SNPs are biological methods based on using microorganisms, enzymes, or fungus; however, these methods need special culture preparation and isolation methods for the fabrication of SNPs. A green chemistry route for the fabrication of SNPs would have many advantages compared with other methods, such as facile fabrication, environmentally friendly, elimination of the need for any special culture preparation and isolation method, low cost, low yields, easy scaling up for the large-scale fabrication of nanoparticles, and not consuming a lot of energy.

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TGA analysis. Thermal characterisation was performed using a thermogravimetric analyser at a heating rate of 10°C min⁻¹ in a nitrogen environment and held for 1 minute at 25°C. Fabricated material of 10 mg was heated in an alumina crucible and the TGA profile was recorded from 25°C to 600°C at a scan rate of 10°C min⁻¹.

Detection of H₂O₂ based on the LSPR-based ROS detection method

The experimental conditions for the LSPR-based ROS detection method using Acacia gum–stabilised SNPs were as follows: 2 mL of different concentrations of H₂O₂ solution prepared using 20 mM phosphate buffer (pH 7.0) was mixed with 3 mL of Acacia gum–stabilised SNPs. Then, the solution mixture was kept at a room temperature of 25 ± 3°C. The change in the optical characteristics of the LSPR-based ROS detection procedure was carried out using a UV-Vis spectrophotometer over the range of 350 to 800 nm at room temperature. In addition, H₂O₂ solution containing the Acacia gum–stabilised SNPs was utilised for TEM observation.

Results and Discussions

Formation of Acacia gum–stabilised SNPs

In this study, SNPs stabilised with Acacia gum were prepared using wet chemical synthesis because it is the most common procedure for the fabrication of uniform nanoparticles of precisely controlled sizes and stable SNPs and their colloidal dispersions in water or organic solvents.35–37 The reduction was performed under a sonication bath or stirrer using non-toxic and environmentally friendly reducing reagent, namely, white sugar, which was responsible for the reduction of silver ions (Ag⁺) to colloidal SNPs (Ag₀), and a non-toxic capping agent, namely, Acacia gum, for size stabilisation of the nanoparticles and to avoid their agglomeration, sedimentation, or loss of surface properties.38 Moreover, NaOH solution was added to fabricate uniform size and shaped nanoparticles.39

Characterisation of Acacia gum–stabilised SNPs

Optical studies. It is well known that the reduction of silver ions into SNPs is commonly followed by a colour change so that the formation of SNPs can be visually observed. Therefore, the change in colour was visually monitored to check for the formation of SNPs and the reaction was stopped when there was no additional change in the solution colour.40–42 Figure 1A shows that the colour of the reaction mixture prepared using a sonication bath or stirrer gradually changed from colourless to brown within 30 minutes due to the formation of SNPs in the solution. In addition, it was observed that there was a difference in the colour of the prepared SNPs using a sonication bath compared with a stirrer.

The effect of the agitation method on the fabricated SNPs was studied using UV-Vis spectrophotometer because SNPs can absorb light in the visible region due to the surface plasmon resonance phenomenon based on their size and shape.43–45 Figure 1B shows the UV-Vis spectra of the Acacia gum–stabilised SNPs prepared under ultrasound and magnetic stirring. It was found that the absorbance peak of SNPs prepared using the sonication bath was higher than the absorbance peak of SNPs prepared using a stirrer. This result indicated that there was a greater reduction of silver ions and a greater formation of SNPs when using a sonication bath, indicating that using ultrasound irradiation can enhance the rate of reaction. In addition, the full peak width at half maximum (FWHM) was calculated, and it was found that the FWHM of SNPs prepared using a stirrer was 90 nm and the large FWHM was due to peak broadening and polydispersity,46 whereas for SNPs prepared using a sonication bath, the FWHM was 75 nm, indicating the formation of uniform SNPs when using a sonication bath.

The maximum absorbance peak of SNPs prepared using the sonication bath was 407 nm, which means there was a slight blue shift compared with the common maximum absorbance peak of the SNPs of 410 to 422 nm.47–49 The results are in accordance with the data reported in the literature, which shows a blue shift of the maximum absorbance peak (408 nm) of starch–stabilised SNPs prepared using D-glucose under sonication irradiation.39 So far, there is no general rule for the shift of the SNP band. However, the shift of the band could be due to the difference in size, shape, and dielectric environment.50,51 Following these results, all further work in this study was performed in a sonication bath for the preparation of Acacia gum–stabilised SNPs.
Morphological characterisation and pore size studies. In this study, the fabricated Acacia gum–stabilised SNPs were characterised using TEM analysis, which can provide information about the morphology and size of fabricated nanoparticles. Figure 2 shows the TEM micrographs of the fabricated Acacia gum–stabilised SNPs, using different magnifications. It was observed that the fabricated SNPs were in the nano range and were well dispersed without aggregation, possessing a spherical shape. The corresponding particle distribution histogram of the fabricated SNPs is shown in Figure 3. It was found that the average size distribution was 10 nm, with a standard deviation of 6 nm.

EDAX analysis. Energy-dispersive X-ray spectroscopy analysis can provide qualitative and quantitative data of the elements of fabricated materials. Therefore, the fabricated Acacia gum-stabilised SNPs were studied using EDAX analysis. Figure 4 shows the EDAX spectrum, displaying the elemental composition of the sample. As can be seen in the middle of the EDAX spectrum, there are 3 peaks located between 2 and 4 keV, related to silver’s characteristic lines K and L, and an optical absorption peak at 3 keV because of surface plasmon resonance. This revealed that the nanostructure was formed of silver. In addition, other elements can be observed on the left part of the EDAX spectrum, namely, carbon (C) at 0.2 keV and oxygen (O) at 0.5 keV. The appearance of carbon and oxygen peaks in the tested samples confirms the presence of the stabiliser. The same result was obtained by other groups.

The EDAX spectrum obtained was used for carrying out the quantitative analysis. It was found that there were high silver contents (58 %) in the examined samples, whereas the contents of carbon and oxygen were 30% and 12%, respectively. The obtained result from the EDAX analysis confirmed the formation of SNPs without impurities.

FT-IR measurement. Fourier transform infrared spectroscopy was carried out to identify the possible Acacia gum responsible for capping, which can increase the stability of SNPs in the colloids. Figure 5 shows the FT-IR spectrum of the fabricated Acacia gum–stabilised SNPs. The intense broad band at 3400 cm⁻¹ can be assigned to O–H, stretching vibrations of carboxylic acid and hydroxyl groups. The absorption band at 1660 cm⁻¹ corresponds to C=O stretching, which is attributed to polycarboxylic. The two bands at 1422 and 1364 cm⁻¹ that are common bands for Acacia gum were not observed. This could be due to the stabilisation of SNPs by hydroxyl bond deformation at the silver metal surface. A similar result was obtained by Rao et al.

Thermal properties of Acacia gum–stabilised SNPs. The thermal properties of Acacia gum–stabilised SNPs were studied by TGA under a nitrogen atmosphere held for 1 minute at 25°C. Ten milligrams of the SNPs was heated in alumina crucibles and the TGA profile was recorded from 25°C to 600°C at a scan rate of 10°C min⁻¹. As can be seen, an initial weight loss can be observed in the temperature range of 30°C to 200°C. This might be due to the desorption of bound water molecules and adsorbed species present in the Acacia gum–stabilised SNPs. The second step (above 200°C) was due to the thermal degradation of the Acacia gum capping around the SNPs to carbon residue.

Evaluation of the Acacia gum–stabilised SNPs as an LSPR-based optical H₂O₂ sensor

The calibration characteristics of H₂O₂ using the Acacia gum–stabilised SNPs as an LSPR-based optical sensor were studied. This was performed by adding 0.001 M H₂O₂ prepared using 20 mM phosphate buffer solution (pH 7.0) to the Acacia gum–stabilised SNPs at a volume ratio of 1:1.5. As can be seen in Figure 7A, bubbles were observed in the solution placed in the
cuvette after 1 minute of adding 0.001 M H₂O₂ solution. The reason for the formation of bubbles in the solution was due to the decomposition of H₂O₂ by the catalytic reaction between H₂O₂, which is a strong oxidising reagent, and the Acacia gum–stabilised SNPs and the subsequent formation of oxygen molecules,61 as can be seen in the following formula that describes the reaction of SNPs with H₂O₂ solution and the transformation of SNPs into silver ions62–64:

\[
Ag + 2H₂O₂ \rightarrow Ag^+ + O₂↑ + 2H₂O \Delta E° = 0.067
\]

The change in the optical characteristics of the LSPR-based optical H₂O₂ sensor with time (1, 4, 8, 12, 16, 20, 30, 40, 50, 60, 70, 80, 90, and 120 minutes) in the visible range (350–800 nm) was monitored. Figure 7B presents the change in the LSPR optical characteristics with time. As can be seen, there was a significant decrease in the absorbance strength at \( \lambda_{\text{max}} \) after 1 minute of adding H₂O₂ solution from 1.880 to 0.872 because of the catalytic reaction between the Acacia gum–stabilised SNPs and 0.001 M H₂O₂ solution. Moreover, there was a red shift of the spectrum band of the Acacia gum–stabilised SNPs to 419 nm after adding the H₂O₂ solution. The reason for the decrease in the absorbance strength and the shift of the LSPR band maximum was because of the destruction of the end capping shell (Acacia gum) resulting in the aggregation of SNPs and a decrease in the distance between the particles. In addition, the catalytic decomposition of H₂O₂ was accomplished with the degradation of SNPs by oxidation of the SNPs by the strong oxidising agent (H₂O₂), resulting in the conversion of Ag⁺ to silver ions (Ag⁺).18,39,65 These results showed that the change of the absorbance strength at \( \lambda_{\text{max}} \) of the LSPR spectrum band can be used as a sensitive signal for fast colorimetric detection of H₂O₂ solution.

**Quantitative determination of H₂O₂**

For quantitative determination of the H₂O₂ concentration using the Acacia gum–stabilised SNPs, different concentrations of H₂O₂ solution (0.5–0.00001 M) prepared using 20 mM phosphate buffer solution (pH 7.0) were added to Acacia gum–stabilised SNPs at a volume ratio of 1:1.5, and the UV-Vis absorption spectra were recorded after 60 minutes of adding H₂O₂ solution to evaluate the calibration characteristics. Figure 8A shows a photograph of the Acacia gum–stabilised SNPs after introducing different concentrations of H₂O₂ solution showing the change in colour of the Acacia gum–stabilised

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**Figure 4.** Energy-dispersive X-ray spectroscopy characteristic spectrum of the fabricated Acacia gum–stabilised silver nanoparticles.

**Figure 5.** Fourier transform infrared spectroscopy transmittance spectrum of the fabricated Acacia gum–stabilised silver nanoparticles.
SNPs from yellow by LSPR absorption to transparent. The change of the solution colour was due to the aggregation of SNPs induced by H₂O₂ solution. A higher concentration of H₂O₂ solution causes a higher aggregation and a colour change of SNPs that can be observed by the naked eye, with the colour change being directly proportional to the amount of H₂O₂ solution added to Acacia gum–stabilised SNP solution.

As there was a change in the colour of the Acacia gum–stabilised SNP solution, the LSPR optical characteristic change with time could be monitored with a UV-Vis spectrophotometer. The relationship between the change of LSPR absorbance strength and the concentration of H₂O₂ solution can be seen in Figure 8B. It is obvious that there was a remarkable change in the LSPR absorbance strength, which depended on the concentration of H₂O₂ solution. This resulted from the catalytic decomposition of H₂O₂ solution inducing the aggregation of the Acacia gum–stabilised SNPs, thus resulting in a higher decrease in the absorbance of the Acacia gum–stabilised SNPs; a similar result was obtained by other groups.

To make sure that the LSPR absorbance strength change was caused by the catalytic reaction between H₂O₂ and the Acacia gum–stabilised SNPs only, distilled water mixed with 20 mM phosphate buffer solution (pH 7.0) was added to the Acacia gum–stabilised SNPs at a volume ratio of 1:1.5, and the experiment was rerun. It was found that there was no change in the LSPR absorbance strength, thus confirming that the decomposition of SNPs was due to adding H₂O₂ solution.

**Linear range and precision**

The kinetic curves at different concentrations of H₂O₂ solution, in the range between 0.5 and 0.00001 M, added to the
Acacia gum–stabilised SNPs with time were studied to evaluate the analytical applicability of the fabricated SNPs as an LSPR-based sensor. Figure 9 shows the relationship between absorbance strength change and time for different H$_2$O$_2$ concentrations. The relative change of the absorbance strength was presented as the ratio $(A_o - A_t) / A_o$, where $A_o$ and $A_t$ are the absorbance of the Acacia gum–stabilised SNPs only and the absorbance of the Acacia gum–stabilised SNPs after adding H$_2$O$_2$ solution at different times, respectively. The ratio $(A_o - A_t) / A_o$ was proportional to the degree of conversion of the catalytic reaction between the H$_2$O$_2$ solution and Acacia gum–stabilised SNPs.

The linear range and precision using Acacia gum–stabilised SNPs as an LSPR-based chemical sensor for the colorimetric detection of H$_2$O$_2$ were investigated. Figure 10 presents the relationship between the change in the absorbance strength and the concentration of H$_2$O$_2$ solution at a reaction time of 15 minutes. A linear response of the Acacia gum–stabilised SNPs as a function of H$_2$O$_2$ solution concentration can be seen, with the linear range of 0.1 to 0.00001 M and a correlation of estimation $R^2$ of .953, thus confirming that the fabricated SNPs are suitable for colorimetric detection of the oxidising reagent (H$_2$O$_2$).

The within-batch and between-batch precision of the procedure was investigated as the relative standard deviation (RSD) by calculating the intraday and interday precision ($n=3$) of the analysis of 0.001 M H$_2$O$_2$ in triplicate analyses (Table 1), and it was found to be between 3.1% and 4.7% for the intraday precision and between 3.5% and 6.4% for the interday precision. Therefore, this method was reproducible for the determination of H$_2$O$_2$ solution.

**TEM imaging for evaluation of the Acacia gum–stabilised SNPs**

The fabricated Acacia gum–stabilised SNPs were examined by TEM analysis after adding H$_2$O$_2$ solution. As can be seen in Figure 11, it was observed that the morphology of the Acacia gum–stabilised SNPs was completely changed after introducing H$_2$O$_2$ solution and the spherical colloids could not be seen, compared with Figure 2. Before adding H$_2$O$_2$
solution, the Acacia gum–stabilised SNPs were stable and dispersed in the dispersion medium, resulting in effective excitation of the LSPR band absorbance. On the contrary, when H$_2$O$_2$ solution was added to the Acacia gum–stabilised SNPs, the nanoparticles were aggregated by the catalytic reaction between SNPs and H$_2$O$_2$ solution, and the aggregation of nanoparticles causes the LSPR band absorbance strength to decrease.$^{63,64}$

Investigation with a real sample

To investigate the actual applicability of the fabricated SNPs for real sample analysis, two different types of liquid milk samples (Almarai and Alsafi) where chosen as real samples; 5 × 10$^{-3}$ and 2 × 10$^{-4}$ M H$_2$O$_2$ solution was added to Acacia gum–stabilised SNPs at a ratio of 1:1.5, and the solutions were subsequently analysed using a UV-Vis spectrophotometer. As can be seen in Table 2, the milk sample without spiked H$_2$O$_2$ solution did not provide any detectable signal, whereas the recovery for the H$_2$O$_2$ solution was found to be more than 97%, with an RSD of less than 6% (n = 3). This result shows that the proposed procedure was suitable for measuring H$_2$O$_2$ solution in liquid milk samples.

Table 1. The intraday and interday precision (n = 3) of the analysis of 0.001 M H$_2$O$_2$ solution in triplicate analyses.

| INTRADAY PRECISION | INTERDAY PRECISION |
|--------------------|--------------------|
| 3.1                | 5.9                |
| 4.7                | 3.5                |
| 4.4                | 6.4                |

Conclusions

In the present investigation, Acacia gum–stabilised SNPs were fabricated through a rapid, simple, eco-friendly, and economically sustainable green method to synthesise SNPs using white sugar as a reducing reagent, Acacia gum as a stabilising agent, and NaOH as a reaction accelerator. The fabricated Acacia gum–stabilised SNPs in this study had an LSPR band at 407 nm and high colloidal stability. Moreover, the chemical composition, morphology, and physical and thermal properties of the fabricated nanoparticles were characterised using different techniques. The results of the TEM micrographs confirmed that the fabricated nanoparticles were in the nano range and that they were spherical with a narrow size distribution and average diameter of 10 nm. The Acacia gum–stabilised SNPs were used for the detection of an H$_2$O$_2$ solution, which was monitored using spectrophotometry. The results demonstrated that the LSPR optical characteristics drastically changed after adding H$_2$O$_2$ to the Acacia gum–stabilised SNP solution depending on the concentration of H$_2$O$_2$, caused by the degradation of SNPs induced by the catalytic decomposition of H$_2$O$_2$. From these characteristics, this LSPR-based optical sensor for the detection of H$_2$O$_2$ using the fabricated nanoparticles was shown to be a fast, simple, and cost-effective method that could be applied as a colour indicator in different applications of industrial, medical, and environmental research.

Figure 10. Calibration curve of H$_2$O$_2$ from 0.1 to 0.00001 M using Acacia gum–stabilised silver nanoparticle–based localised surface plasmon resonance colorimetric sensor.

Figure 11. Transmission electron microscopy micrographs of Acacia gum–stabilised silver nanoparticles after introducing 0.001 M hydrogen peroxide solution.
Table 2. Recovery experiments for determination of H$_2$O$_2$ in real sample (n=3).

| SAMPLE     | FOUND (M) | SPIKED (M) | FOUND (M) | RECOVERY (%) | RSD (%) |
|------------|-----------|------------|-----------|--------------|---------|
| Milk (Almarai) | Not detected | 5 × 10$^{-3}$ | 4.9 × 10$^{-3}$ | 98           | 4.7     |
| Milk (Alsafl) | Not detected | 2 × 10$^{-4}$ | 1.95 × 10$^{-4}$ | 97.5         | 5.3     |

Abbreviation: RSD, relative standard deviation.

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