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Preparation of red and grey elemental selenium for food fortification

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SHORT COMMUNICATION

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ABSTRACT

In recent years, the importance of nanomaterials in food science, medicine, etc. has been increasing quickly. Herein, organic and inorganic red selenium nanoparticles synthesised by the reduction of sodium selenite with chemical and biological reducing agents. Grey hexagonal form in aqueous and powder was assembled at a high temperature of 85 °C for 10 min. Also, selenium enriched yogurt powder was made that contained about 2,000 mg kg^{-1} selenium, 93.8% of which is in nano form with a size of 50–500 nm. The synthesised nanoparticles were characterised by Dynamic Light Scattering Particle Size Analyzer (DLS), X-ray Diffraction Analysis (XRD), Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). The prepared SeNPs could be promising additive for a wide range of applications.

KEYWORDS

selenium nanoparticles, red, grey, nano granules, yogurt

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1. INTRODUCTION

Selenium is an efficient element in various biochemical pathways and physiological functions. In nature, a wide range of selenium compounds can be found ranging from simple inorganic forms (selenite, selenate, selenide and elemental selenium) to complex biogenic compounds including selenoenzymes and selenoproteins. Generally, selenium is obtained from food, but in some geographic areas that are poor in selenium content or in cases of some diseases, the daily intake of selenium has to be increased. Actually, selenium deficiency affects anywhere from 500 million to 1 billion people worldwide according to a review (Prabhu and Lei, 2016), well documented especially in China and New Zealand. Selenium content in most parts of Europe is considerably poorer than in the United States. The average intake of selenium in Eastern Europe is lower than in Western Europe (Prabhu and Lei, 2016). Therefore, a regulation (EC) No 1925/2006 of the European Parliament and of the Council (EC, 2006a) included that selenium (sodium selenate, sodium hydrogen selenite and sodium selenite) may be added to foods. Basically, health benefits of selenium including maintenance of normal hair, maintenance of normal nails, protection against heavy metals, maintenance of normal joints, maintenance of normal thyroid function, protection of DNA, proteins and lipids from oxidative damage and maintenance of the normal function of the immune system are authorised by the European Food Safety Authority (Regulation (EC) No 1924/2006) (EC, 2006b) and EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010. The recommended daily dose (RDA) for adults is from 55 to 70 μg/person per day, and the maximum level of selenium is 300 μg/person per day according to the regulation (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2014). Actually, selenium bioavailability varies depending on several factors, such as chemical forms, dose, solubility, other dietary components and physiological status. Selenate and selenite have an oxidant mode of action in living organisms and are very toxic. Selenium exposure can result in either acute or chronic health problems.

Intake of 250 mg selenium (potassium selenate, sodium selenate and sodium selenite) as a single dose or in multiple doses of 25–30 mg can result in acute toxic effects in humans (EMA/CVMP/187590/2015). Also, 2–5 mg kg^{-1} selenium in feed can result in subclinical toxicity in food-producing animals, and 25 mg kg^{-1} in feed gives rise to acute toxicity in laboratory animals (EMA/CVMP/187590/2015, European public MRL assessment report). Acute exposure is explained by selenium neurotoxicity, while chronic exposure is explained by the toxic effect on endocrine function, especially in the synthesis of thyroid hormones (Vinceti et al., 2001; 2014). However, the elemental form of selenium has lower toxicity (Jia et al., 2005) and higher bioavailability (Kojouri et al., 2012) compared to other inorganic forms. Therefore, in recent years, the synthesis of selenium nanoparticles (SeNPs) and its application has been investigated in food supplementation (Skalickova et al., 2017), food packaging systems (Vera et al., 2016) and nanomedicine (Hosnedlova et al., 2018) due to its functional properties, including high antioxidant (Wang et al., 2007), anticancer (Pi et al., 2013), immune-stimulatory (Kojouri et al., 2012), detoxification (Cogun et al., 2012; Prasad and Selvaraj, 2014), antibacterial (Menon et al., 2019), antifungal (Fardsadegh et al., 2019) and antidiabetic (Zhao et al., 2017) effects. For example, SeNPs based packaging showed higher antioxidant properties than non SeNPs packaging, as it extended shelf-life and inhibited the oxidation of food (Vera et al., 2018). Basically, there are safety concerns on the application of nanoparticles in food packaging due to the possible migration of nanoparticles from packaging into the food, with potential human

toxicological effects. Selenium nanoparticles may be the perfect additive to solve these problems together.

2. MATERIALS AND METHODS

2.1. Chemicals

Sodium selenite (Na_2SeO_3) and L-ascorbic acid were purchased from VWR International Ltd. (Lutterworth, Leics. UK). Yogurt starter culture (Lyofast Y 250) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were obtained from SACCO Srl (Italy).

A modified chemical reduction method was used. A stock solution of 500 mg dm^{-3} and 10,000 mg dm^{-3} sodium selenite and 10 g dm^{-3} and 100 g dm^{-3} ascorbic acid solution was made for the preparation of red and grey elemental selenium.

2.2. Characterisation

The particle size and size distribution of the produced SeNPs were measured by dynamic light scattering (DLS) using the Malvern Mastersizer 2000 particle size analyser. The X-ray powder diffraction patterns (XRD) of the samples were recorded on a Rigaku-Oxford Diffraction SuperNova Dual source diffractometer with Cu Kα source (λ = 0.15406 nm). The morphology was analysed by Hitachi S-4300 scanning electron microscope (SEM) and transmission electron microscope (TEM). Also, selenium concentration was measured by flame emission atomic absorption spectrometer (Thermo ICE 3000) and atomic fluorescence spectrometer (PSA Thermo, Excalibur). The synthesised selenium nanoparticles in the fermentation process purified for characterisation of morphology. For preparation and purification, 20 mL of 10,000 mg dm^{-3} sodium selenite stock solution was added into 980 mL MRS broth, then 10 mL of fresh bacterium culture was added, and the mixture was incubated at 37 °C for 24–36 h. The culture was centrifuged at 6,000 rpm for 15 min, and the pellets were washed with water. Finally, cells were lysed by hydrochloric acid 37% (m/m) for 5 days at room temperature. The mixture was centrifuged at 6,000 rpm for 15 min, washed with water and filtered by vacuum filtration (Prokisch and Zommara, 2011).

3. RESULTS AND DISCUSSION

3.1. Preparation of red and grey elemental selenium

An aqueous solution of selenium nanoparticles was prepared by the reaction of 500 mg dm^{-3} sodium selenite and 10 g dm^{-3} ascorbic acid solutions. Sodium selenite and ascorbic acid solutions were mixed in a 1:1 vol ratio from stock solutions in a plastic tube at room temperature for 30 min (Fig. 1). The mixtures were allowed to react with each other in the concentrated form until a colour change was observed from colourless to red colour. Selenium nanogranules were prepared by precipitation. In this case, 10,000 mg dm^{-3} sodium selenite stock solution was mixed in equal proportions with 100 g dm^{-3} ascorbic acid solution, and the mixture was kept at room temperature for 2 h. The precipitation started after 30 min, and at the end of the reaction time, nanogranules were filtered by a paper filter and washed with alcohol and distilled water

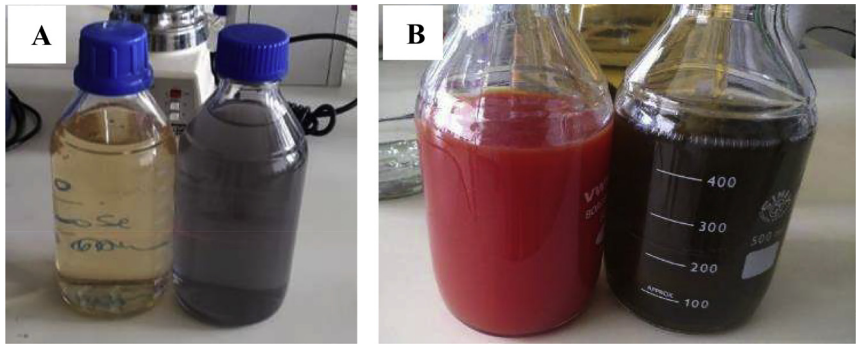

Fig. 1. Red and grey elemental selenium nanoparticles, 20 (A) and 200 mg dm^{-3} Se (B)

three times. Red selenium nanogranules were kept at 4 °C until dried (Fig. 2). Grey-SeNPs were converted at the temperature of 85 °C for 10 min from previously prepared red selenium aqueous solution and nanogranules. Grey nanogranules were dried at room temperature. These processes are shown in Figs 1 and 2. Finally, red and grey selenium nanogranules were ground using a nano grinder. With this method, approximately 5 g red and grey selenium pure nanopowder can be obtained from 1,000 mL of solution.

3.2. Preparation of selenium enriched yogurt powder

Selenium (200 mg dm^{-3}) as sodium selenite and yogurt starter culture were added into 1.5% skimmed milk, and incubated at 37 °C for 24–36 h. At the end of the making period, yogurt colour turned red and 10 mg L^{-1} ascorbic acid was mixed with the yogurt. The yogurt mixture was kept at room temperature for 30 min, then the dried in a freeze-dryer and ground (Fig. 3). Approximately 120 g of selenium-enriched yogurt powder could be obtained from 1,000 mL of milk. The selenium-enriched yogurt powder contains about 2000 mg kg^{-1} selenium, 93.8% of which is in nano form.

3.3. Characterisation

The particle size distribution of SeNPs was measured by a particle size analyser after preparation by chemical and biological methods. The biosynthesised SeNPs, accumulated intracellularly in

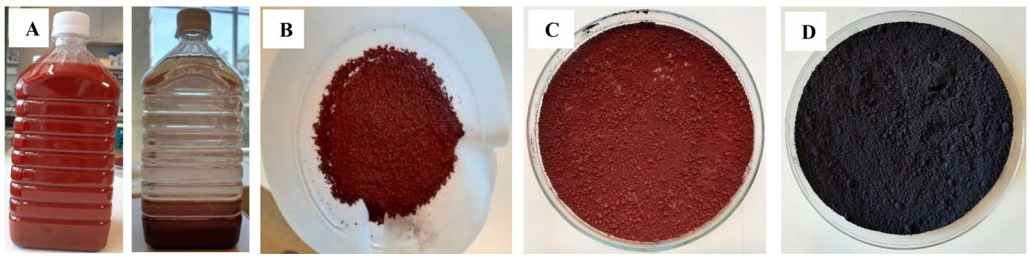

Fig. 2. Schematic illustration (A–D) of the preparation process of red and grey selenium nanopowder

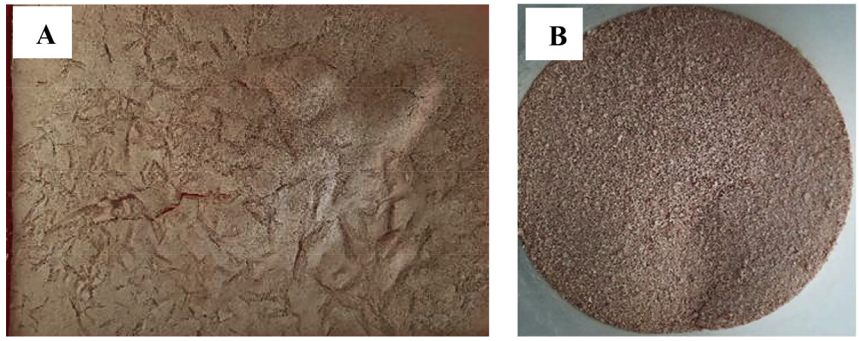

Fig. 3. Dried (A) and ground (B) selenium enriched yogurt powder

bacteria, had size ranging 50–500 nm (Fig. 4). The size of chemically synthesised SeNPs increased from 100 nm after 10 min to 100 µm after 30 min of mixing ascorbic acid and selenite with continuous stirring (Fig. 5). This result showed a direct relationship between an incubation time and the particle size. In the literature, similar results were observed for the biological synthesis of selenium nanoparticles. For example; *Bacillus mycoides* mediated selenium nanoparticles had an average diameter of 50–100 nm and 50–400 nm after 6 h and 48 h of the incubation period, respectively (Lampis et al., 2014) and size of *Azadirachta indica* leaves extract mediated nanoparticles increased from 153 nm to 287 nm after 5 and 10 min of reaction period, respectively (Mulla et al., 2020).

The biologically and chemically synthesised red selenium nanoparticles are amorphous in shape that was indicated by XRD and SEM analysis. The XRD pattern of the chemically synthesised red selenium sample was broader with no sharp Bragg reflections (Fig. 6A). Thus, the data indicate the amorphous nature of the synthesised red-SeNPs, in accordance with findings of Song et al. (2006) and Kora and Rastogi (2016). The amorphous shape of individual and clustered nanoparticles was also confirmed by SEM imaging after purification from the bacterial mass (Fig. 6B).

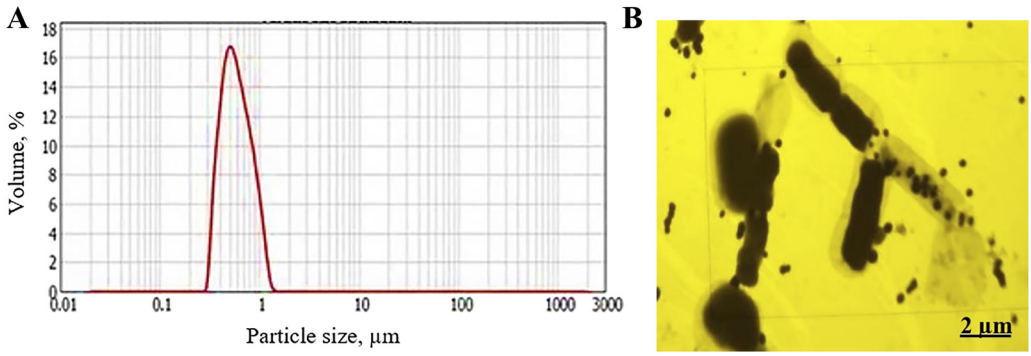

Fig. 4. The particle size distribution of the biosynthesised SeNPs (A) and TEM image of bacteria with SeNPs (B)

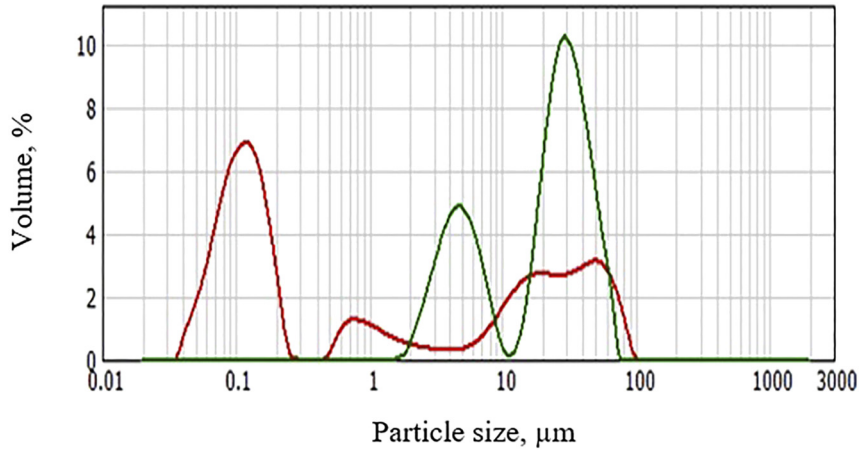

Fig. 5. The particle size distribution of the chemically synthesised SeNPs; 10 min (red curve), and 30 min (green curve)

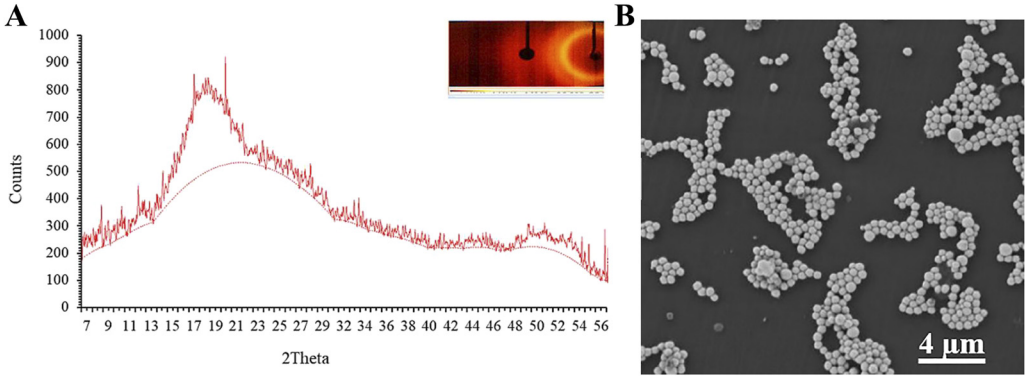

Fig. 6. XRD pattern with corresponding selected area electron diffraction (SAED) of the chemically synthesised red-SeNPs (A), SEM image of the biosynthesised red-SeNPs (B)

Grey hexagonal structure (grey-SeNPs) rapidly transformed from these prepared red amorphous SeNPs in liquid and wet powder at 85 °C for 10 min (shown in Fig. 2). The same results showed that the particles became more crystalline at 50 °C for more than 3 weeks (Hageman et al., 2017), at 130 °C for 6 h (Dwivedi et al., 2011) and 180 °C for 12 h (An and Wang, 2007). Figure 7 shows the XRD pattern and the SEM image of the converted crystal structure from the chemical and biological synthesis, respectively. Sharp and narrow peaks were noticed and impurity peaks were not observed, suggesting high purity and well crystallised SeNPs formation. The selenium peaks centred at 2θ of 23.5°, 29.7°, 41.4°, 43.6°, 45.4°, 51.8°, 55.9°, 61.5°, 65.3° and 71.5° were attributed to the (100), (101), (110), (102), (111), (201), (112), (202), (210) and (113) reflections of the pure hexagonal phase of selenium crystal. The chemically synthesised SeNPs were successfully formed with hexagonal structure, and the lattice

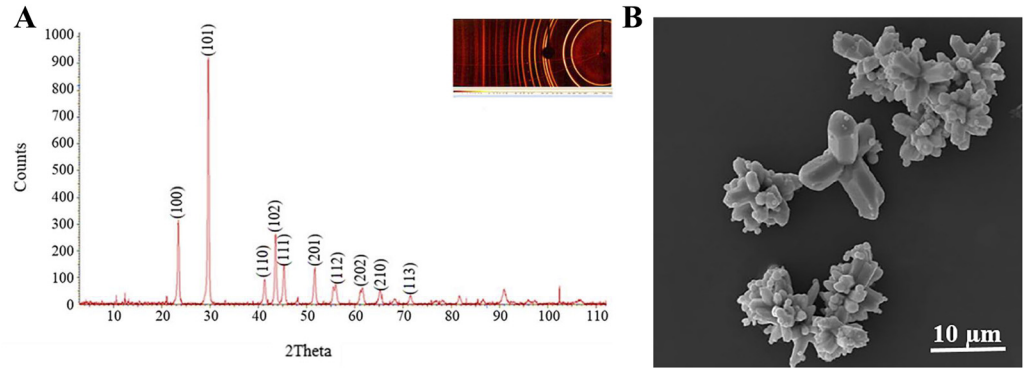

Fig. 7. XRD pattern with corresponding SAED of the chemically synthesised grey-SeNPs (A), SEM image of the biosynthesised grey-SeNPs (B)

constants were $a = 4.36$ Å and $c = 4.95$ Å as per JCPDS card No. 06-362 standard (Dwivedi et al., 2011; Senthil kumaran et al., 2011; Srivastava and Mukhopadhyay, 2015).

4. CONCLUSIONS

Organic red amorphous selenium nanoparticles with sizes ranging 50–500 nm were synthesised by bacterial fermentation process. However, inorganic red amorphous selenium nanoparticles with bigger sizes (100 nm to 100 µm) in aqueous and powder form were synthesised by the reaction of low and high concentrations of sodium selenite and ascorbic acid, respectively. This method is novel, simple and rapid, and suitable to prepare large quantities of nanogranules. Also, grey selenium nanoparticles, a thermodynamically stable form, assembled at a high temperature. The chemically synthesised red and grey elemental selenium nanoparticles could be used in food supplementation and food packaging as functional additives. Also, making selenium-enriched yogurt powder is a unique method that combines the synthesis of selenium nanoparticles and the production of yogurt at the same time, while not requiring special storage conditions.

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