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Green synthesis optimization and characterization of selenium nanoparticles using aqueous extract of peel bitter orange (*Citrus aurantium*)

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ABSTRACT

Background: Bitter orange is a citrus fruit rich in bioactive compounds such as flavonoids, which provide antioxidant and antimicrobial properties. Often, its peel is used in the food industry. This study demonstrated that dried bitter orange peel extract is a reducing agent for the rapid and straightforward synthesis of nano-sized selenium nanoparticles.

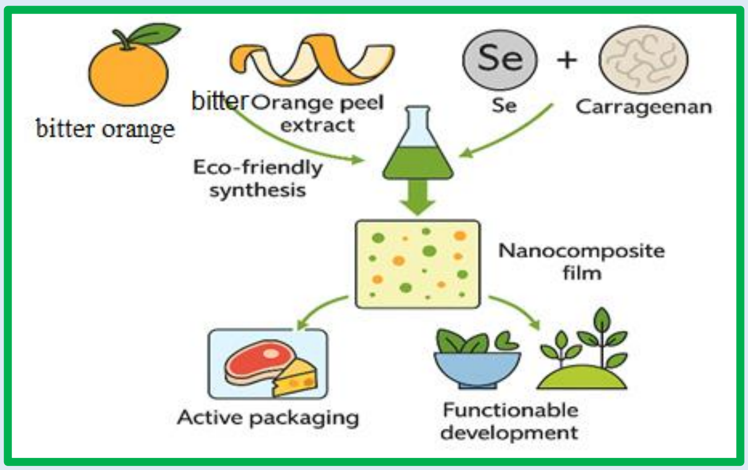
Objective: This study aimed to develop sustainable technology for synthesizing selenium nanoparticles from the aqueous extract of bitter orange peels, which were used as reducing and capping agents.

Results: The results of nanoparticles, which were studied in this experiment using UV-Visible spectroscopy, displayed that surface Plasmon resonance was centered at 289 nm. This experiment also concluded an absorption of 1.098. The FT-IR spectroscopy observed the presence of ascorbic acid, phenolic compounds, and a hydroxyl group. X-ray diffraction (XRD) analysis revealed a spherical crystal structure. The highest diffraction intensity was observed at the 100-crystal plane at a 2θ angle of 27.691°. The peak width at this position corresponded to a crystallite size of approximately 18 nm. This concluded that all particles were within the nanoscale range. The scanning electron microscopy (FE-SEM) results concluded that the nanoparticles were homogeneous in size and, after preparing a selenium solution, the crystal ball diameter ranged between 29.24 and nd 59.14 nm. The treatments coated with composite and straightforward carrageenan films developed a distinctive texture and aroma and maintained their shape, color, and overall acceptability throughout the refrigeration period.

Novelty: This study demonstrates that using bitter orange peels may be a natural source of bioactive compounds such as phenols and flavonoids. Furthermore, selenium particle preparation using green, environmentally friendly methods within a natural polymer matrix of carrageenan may offer vigorous antioxidant activity. This produces nano-films, which may serve as innovative/active packaging materials that maintain the quality and safety of functional food products. This enhances the biological value of active ingredients while improving their biological efficiency through the nano system. Recycling fruit waste of bitter orange peels and using renewable natural materials like carrageenan offers additional green technologies without using solvents or harmful compounds. This will propel the development of sustainable goals in clean production and waste reduction.

Conclusion: Bitter orange peel extracts act as reducing agents for the rapid and straightforward synthesis of nano-sized selenium nanoparticles. This provides sustainable and economic benefits to produce functional foods.

Keywords: bitter orange peel, selenium oxide, carrageenan, green synthesis, particle characterization, Food processing (burger).



INTRODUCTION

The interest in nanotechnology and nanoscience research has increased significantly in recent years, earning it the nickname "small science." Nanoparticles, the basic building blocks of nanotechnology, range from 1 to 100 nanometers and are made of carbon, metals, metal oxides, or organic materials. They were characterized by their high surface charge, high absorption, large surface area, sensitivity, stability, and strength [1-2]. Nanotechnology is rapidly evolving as it holds great promise in the food, medical, and mechanical industries compared to large particles of the same composition. Nanoparticles exhibited higher biological and chemical activities, penetrability, catalytic behavior, enzymatic reactivity, and quantitative properties due to their larger surface area and increased mass transfer rates [3]. With this, nanoscale food additives such as chelating agents can be enhanced by the effectiveness of nanoparticles. The nutritional composition of food products can influence flavoring, preservatives, antimicrobial sensors, and packaging materials. Nanoscale food additives can enhance flavor, texture, and shelf life by identifying food pathogens that can be utilized as indicators for food safety and quality [4- 5].

Food additives (color and flavor) and nutritional supplements (proteins, vitamins, and antioxidants) were packaged to create nanocapsules that could protect functional foods. This allowed for the masking of off-flavors while controlling the release of nutrients through intelligent/thoughtful systems. This has allowed electrospun nanofibers to receive widespread attention as food packaging materials or films [6-7].

Plant extracts contain enzymes, cofactors, flavonoids, and proteins, which allow them to act as reducing agents [8-9]. Prepared from specific plant parts such as dried leaves, seeds, flowers, and bark, synthesized nanoparticles have helped address growth in technology and the environment [10]. Nanoparticles prepared using green methods or green synthesis typically have high catalytic capacity due to their high surface area and ability to increase reactivity by producing reactive oxygen species, leading to increased toxicity in bacteria [11]. Most researchers have indicated that selenium nanoparticles (SeNPs) are acceptable for use. Therefore, they were recommended for their low toxicity, high stability, and possible manufacturing through various physical, chemical, and biological processes [12-13]. This study used selenium solution for green synthesis by using fruit extracts as an environmentally friendly method, including various fruit extracts, including broccoli, grapes, oranges, pomegranates, and tomatoes, to synthesize selenium nanoparticles [14]. Orange and bitter orange juices were effective reducing agents and stabilizers for green synthesis of SeNPs [15-17].

MATERIALS AND METHODS

Preparation of selenium nanoparticles and their detection by chromatography: 3 g of orange peel extract was mixed with 100 mL of deionized distilled water and placed in a 250 mL glass beaker. The mixture was stirred continuously for at least 30 minutes at 50°C ± 5 °C using a hot plate with a magnetic stirrer. 100 mL of 0.5 mM SeO2 solution was added, and the final solution was placed on a heating device with continuous stirring for≥5 hours at a temperature of 70°C ± 5, until the color of the extract changed and the ruby red color remained. This characteristic is attributed to the SPR phenomenon, a property possessed by many metals, including Se, Ag, and Zn, resulting from the diameter of the particles [18].

Characterization of Selenium Nanoparticles

Spectroscopic analysis using a UV-Vis spectrophotometer: The UV-Vis spectrophotometer was used to determine the optical properties of selenium nanoparticles produced from the aqueous extract of bitter orange peels. One mL of the previously prepared extract was taken, and the sample was placed in a UV-Vis spectrophotometer at 190-400 nm wavelength. The absorbance values of the selenium oxide particles were recorded [19].

FTIR Spectroscopy: Infrared spectroscopy was used to study the molecular vibrations of selenium nuclei with a spectral range of 400-4000 cm to identify the active groups in the sample. Spectra were measured at a laboratory temperature of 24°C, using drops of the solution placed on the device's cell [19].

X-ray diffraction (XRD) analysis: The solution extracted from selenium oxide nanoparticles was measured at the Ministry of Industry and Minerals—Petrochemical Department. This is a common analytical technique for analyzing nanoparticles' molecular and crystalline structures, qualitatively identifying different compounds, and quantitatively determining [19].

Field emission scanning electron microscope (FE-SEM): The examination used a FEI 450 device, manufactured in the Netherlands, to determine the dimensions and shape of the nanoparticles of the orange peel extract. The nanoparticle solution was prepared for examination at 1% concentration by dissolving 1 g of the aqueous extract of the nano orange peels in 1 mL of petroleum ether. Then, the volume was increased to 100 mL in a volumetric flask. The sample was then filtered by using Whatman No. 21 filter paper. A few drops of the filtrate were placed on the surface of a glass slide and allowed to dry at room temperature. Then, the sample was examined using a scanning electron microscope (FE-SEM) [20-21].

Preparation of Carrageenan Composite Films: The composite films were prepared according to the method mentioned by [22] with some modifications.

Casting and Drying the Membranes: The method described by [23] was followed, with some modifications, to prepare and mold the membrane for casting and molding.

Packaging of Burgers: The burgers were prepared and packaged with composite and straightforward films made from carrageenan and enriched with nano-peel extracts. They were maintained in a refrigerator at 4°C. The necessary tests were conducted before and during the refrigerated storage periods for 0, 5, 10, and 15 days.

Appearance Sensory Evaluation: Ten evaluators from the Department of Food Sciences evaluated the prepared samples after packaging, before cooking. The evaluation averages were taken for the attributes mentioned in the sensory evaluation form [24]. Sensory quality tests were conducted on the packaged and unpackaged samples in the refrigerator for 0, 5, 10, and 15 days, including general appearance, odor, texture, and apparent color.

RESULTS AND DISCUSSION

Detection by colorimetric contrast: The bitter orange peel extract turned ruby red instead of yellow following the addition of the Se solution, which confirms the synthesis of ultra-small selenium nanoparticles. This phenomenon is attributed to phenolic compounds, polymethyl flavonoids, coumarins, and ascorbic acid, which act as reducing agents for SeO_2. This is consistent with the results obtained by [25-26], who observed a color change from yellow to ruby red in the bitter orange peel extract after adding the selenium solution stored at a 24°C laboratory temperature.

Ultraviolet–visible spectrophotometry (UV-Vis): A UV-Vis spectrometer was used to detect the crude extract of orange peels and the selenium nanoparticles synthesized from them at wavelengths of 190-400 nm. Figure 1 shows the strongest absorption peaks within the spectrum at 190-400 nm, which falls within the ultraviolet range. The highest absorbance was 289 (1.098), which falls within the electromagnetic spectrum and has a 100-400 nm wavelength. This appeared shorter than the visible wavelength and longer than X-rays. A slight absorption in the 400-600 nm region was observed from the absorption spectrum curve. This phenomenon occurred due to dipole oscillations that form when an electromagnetic field within the visible range couples to the collective oscillation of conduction electrons. This phenomenon was essentially a unique optical property of nanometer-sized metals.

The optical measurements of Se nanoparticles were performed using dried bitter orange peel extract and a UV-Vis spectrometer, consistent with those obtained from [27]. When the wavelength was measured, it was found to be located at 275 nm. The peak recorded at a wavelength of 289 nm was due to the Se-NPs' surface plasmon resonance, which was determined to be their distinctive peak. Several variables, including particle size, may affect the material's absorbance, surface roughness, and crystalline structure. Prepared nanoparticles were more responsive and effective in the ultraviolet region and can thus be used in this region for further applications. These results agreed with those of [26]. UV-

Vis spectroscopy of gelatin coatings reinforced with nanoselenium from orange peels showed the highest absorption peak at a wavelength of 241 nm, with an absorbance of 3.93.

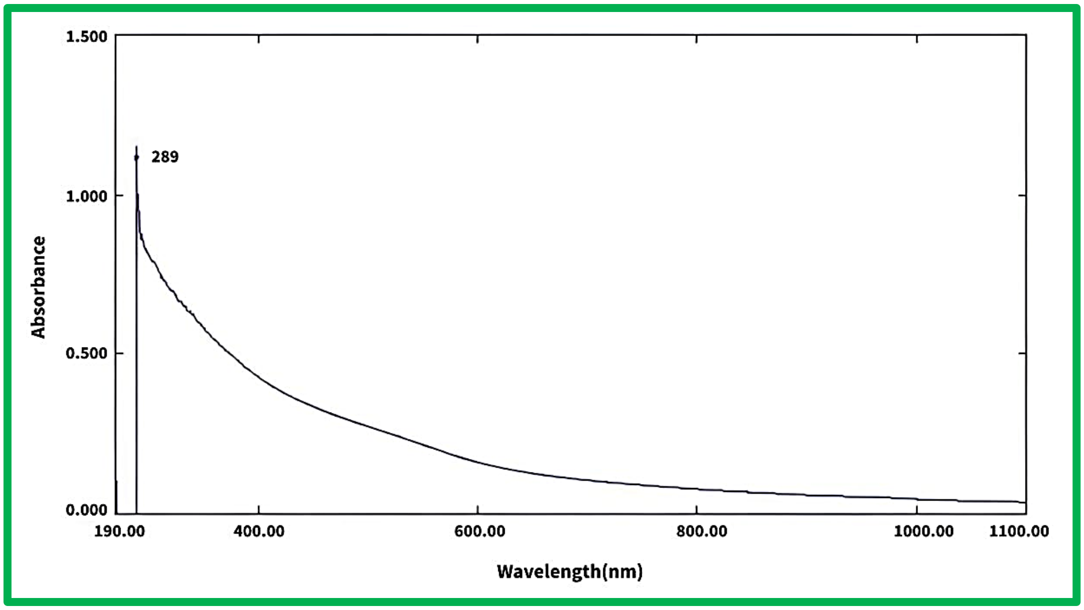


Figure 1. The Ultraviolet–Visible spectrophotometry absorption spectrum of bio-synthesized selenium nanoparticles from orange peels.

Fourier Infrared Spectroscopy (FT-IR): Figure 2 shows the Fourier Transform Infrared (FTIR) spectrum of the nano-selenium dioxide using orange peels. Several prominent absorption peaks appeared within the broadest range at 600-750 cm^{-1}, corresponding to the stretching vibration mode of selenium oxide. The broad absorption band also indicated that the selenium oxide powders were nanocrystals.

In Figure 2, the absorption band centers at 3429.20 and 3409.91 cm-1, which indicates (O-H) stretching vibrations of the phenolic compounds. The weak band is 1631.67 cm-1, corresponding to the(H-O) band of H2O. Therefore, the H bending vibration mode was introduced due to the moisture absorption while preparing the FTIR in open air.

This demonstrated the effect of H_2O on the structure and indicated the presence of hydroxyls in the raw material. The broad absorption band at approximately 663.47 cm^{-1} was attributed to band stretching vibrations. The saw tooth absorption bands in the 1100-1500 cm^{-1} region were assigned to symmetric and asymmetric C-O stretching vibrations. The band intensity was weakened, indicating the presence of ultrafine forces that favor strong physical absorption of H_2O and CO2. FTIR showed that the phenols and ascorbic acid in the bitter orange peel extract were responsible for synthesis and stabilization. The functional groups, absorption peaks, and vibrational patterns of the nanoparticles were identified using Fourier transform infrared spectroscopy. These results were consistent with a study by [26] investigating the biomolecules responsible for reducing and stabilizing the FTIR SeNps spectrum of orange peel extract. Typical peaks 2995.45 and 663.51 cm^{-1} were observed in the orange peel extract and selenium FTIR spectra. The strong band at 2995.45 cm^{-1} in the FTIR was found to have an OH stretch in alcohol and phenolic compounds.

The results are also consistent with a study conducted by [28], where the biomolecules that stabilized and reduced the FTIR SeNps of guava leaf extract and nano Se. Peaks at 3230, 1762, and 1674 cm^{-1} were observed. The strong band at 3230 cm^{-1} in the infrared spectrum was found to have OH stretching in alcohols and phenolic compounds. The strong absorption peak at 1762 cm^{-1} was attributed to the C=O bond stretching of the five-membered lactone ring of ascorbic acid. The peak at 1674 cm^{-1} was due to the C=C bond stretching vibrations. Additional peaks were observed in SeNps in the 1200-1500 cm^{-1} region related to the CH_2. The strong absorption peak 663.51 cm^{-1} is attributed to the band stretching vibrations, and the additional peaks 1000-1500 cm^{-1} are related to C-O.

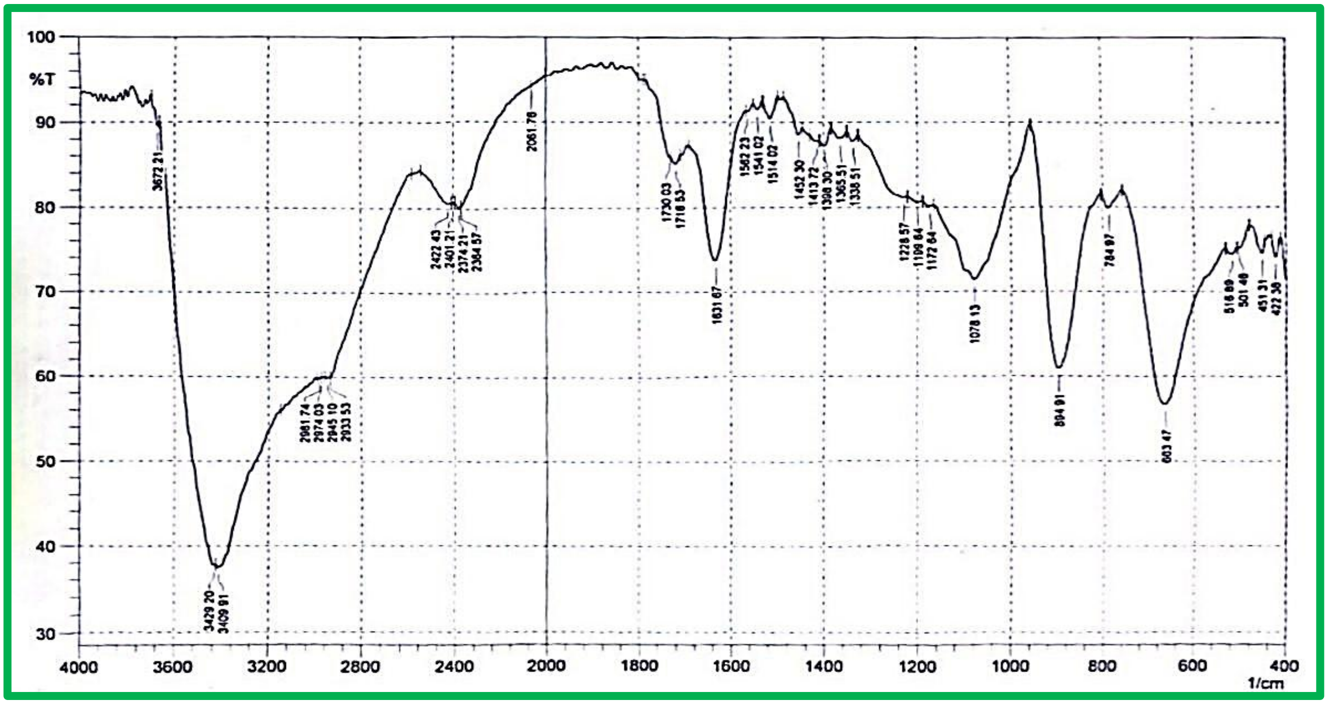


Figure 2. Fourier Infrared (FT-IR) spectroscopy results for selenium dioxide nanoparticles using bitter orange peels.

X-ray diffraction (XRD): Figure 3 investigated nanoparticles as more regular clusters with few impurities, high purity, a mixture of selenium particles, and repeating units from the plant. No peaks containing impurities appeared due to the reaction between selenium oxide and the aqueous extract to manufacture selenium nanoparticles. The crystallite size of the obtained Se nanoparticles was calculated according to the Scherrer Equation, which calculates the size of nanoparticles when the angle of incidence is known and the peak width of one of the diffraction pattern peaks, using the width at 2θ from peak 27.691°(100), is 18 nm.

The results of their study were consistent [28] regarding the synthesis and characterization of Se nanoparticles using plant biomolecules. The X-ray diffraction involving the examination of selenium nanoparticles showed that selenium was prepared by biological methods using guava leaf extract. The crystalline nature and purity of the nanoparticles were obtained from diffraction peaks observed at 2θ = 23.3° (100), 29.6° (101), and 43.5° (120). The results were similar to those achieved by [26], who prepared selenium-enriched gelatin coatings for nanoparticles. The study appeared to illustrate the effects of preserving some meat products. The x-ray diffraction examination showed that the diffraction peak versus angle was 2θ = 32.855°. The values of the crystal planes ranged between 13.835° and 103.019°, which proved that all particles were nanosized and that diffraction values provided information about the amount of defects in the crystal, as the diffraction intensity values in this sample were minimal, indicating a good crystalline order in the nanoparticles without any impurities.

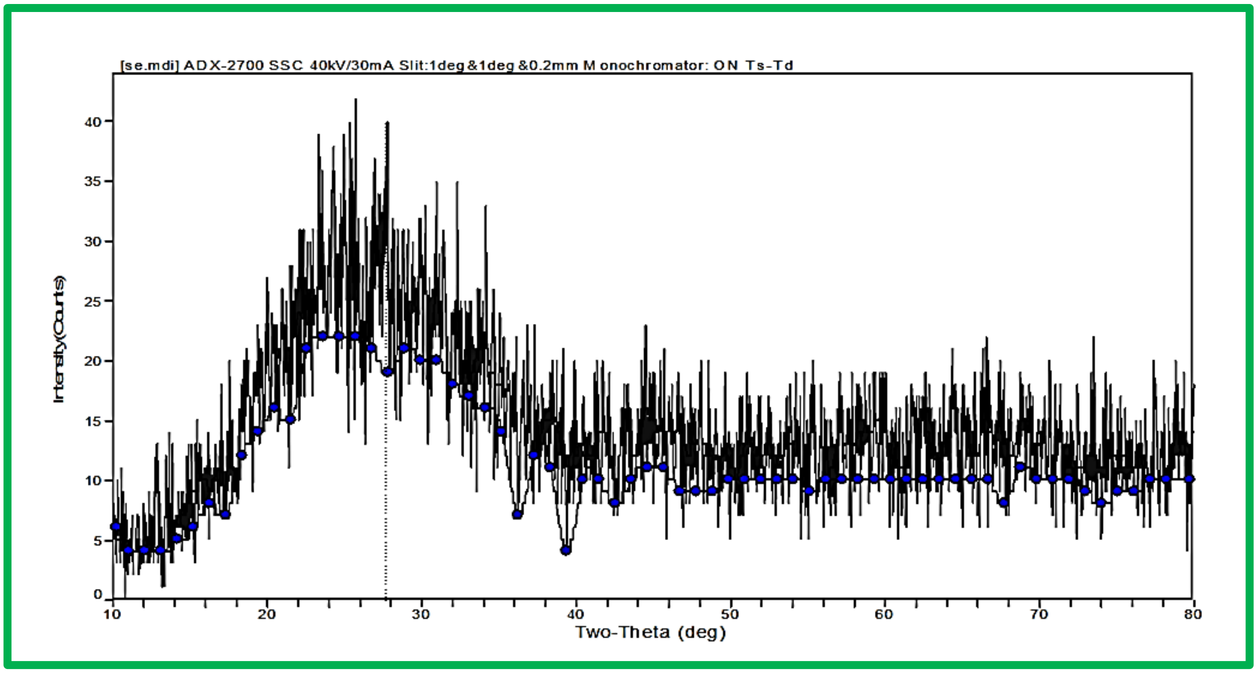


Figure 3. X-ray diffraction of selenium nanoparticles prepared by green methods in bitter orange peels.

Field emission Scanning Electron Microscope (FE-SEM): Figure 4 depicts the shape and size of the synthesized selenium nanoparticles, which were spherical and non-agglomerated. Their nanoparticle sizes ranged between 29.24 and 59.14 nm. The results were consistent with [29], where the sizes of the biosynthesized selenium nanoparticles from ivy ranged between 25.68 and 141.17 nm. While a study conducted by [26] showed a rapprochement, when the sizes of biosynthesized Se nanoparticles ranged from 8.73 to 92.5 nm, the current plant (18-50 nm) average diameter was 27.10 nm. This concluded that black currant polyphenols affect the particle size and the final nature of SeNPs, which depend on the pH and molarity of the Se solution and the reaction temperature. The use of raisins in the synthesis of green was attributed to their content of phenols, sugar, iron, calcium, potassium, and some vitamins.

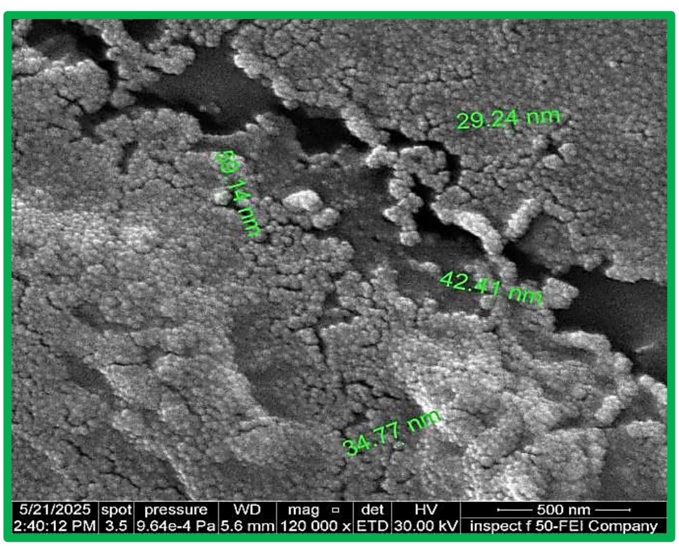


Figure 4. Field emission Scanning Electron (FE-SEM) Microscope Images of selenium dioxide nanoparticles were synthesized using bitter orange peels.

Sensory Evaluation: Table 1 depicts the sensory characteristics of burgers coated with composite and straightforward carrageenan films reinforced with selenium nanoparticles using orange peels and the uncoated control treatment. The results show changes in the sensory characteristics of the uncoated and coated burgers throughout the storage period at 4°C and refrigerated. The effect of coating with composite and straightforward films on the overall quality of burgers is also shown. It also illustrates that coating with composite films of carrageenan can reinforce the selenium particles prepared using green methods on the first day, at time zero. All samples achieved identical approximate evaluation scores before refrigeration, but decreased with increasing storage time. The decrease was slower in the composite film treatment compared with the control sample. This was concluded using the treatments coated with composite and simple carrageenan films. At the same time, they acquired a distinctive texture and aroma, maintained their overall shape and color throughout the refrigerated storage period. This is due to the characteristics of the membranes' barrier and mechanical properties, the role of phenolic and flavonoid compounds in the extracts, and the enhanced efficiency of the prepared membranes, as confirmed by [30-32]. Sensory attributes of food, such as flavor, appearance, and texture, were key for the final acceptance of food products. When considering practical applications, attention must be paid to the desired properties of edible films such as adhesion, cohesion of the food surface, and their ability to withstand handling and storage [33-36]. The acceptance of burger samples coated with carrageenan fortified with selenium nanoparticles may be due to the reduced oxidation reactions in meat during storage and the reduced presence of microorganisms that lead to meat spoilage [37-39].

Table 1. Sensory evaluation of the appearance of wrapped and unwrapped burgers during four periods of refrigerated storage at 4°C.

Treatment	Storage	*Consistency*	*smell*	*color*	*General appearance*	*Final grade*
1 T	0	5	5	5	5	20
	5	4	4	4	4	16
	10	3	3	2	3	11
	15	2	2	2	2	8
2T	0	5	5	5	5	20
	5	4	4	5	5	18
	10	4	4	3	4	15
	15	4	3	3	3	13
3T	0	5	5	5	5	20
	5	5	5	5	5	20
	10	5	4	4	5	16
	15	4	3	4	4	15
L.S.D.		1.65 *	1.68 *	1.65 *	1.74 *	3.219 *
*** P≤0.05**						

T1: Control, T2: Carrageenan Coating only, T3: Carrageenan coating enriched with selenium nanoparticles.

CONCLUSION

This study demonstrated the feasibility of preparing Se nanoparticles using the aqueous extract of bitter orange peel as a reducing agent, characterized by color change. Molecular identification results (UV-Vis) were confirmed with the formation of nanoparticles. FE-SEM revealed the spherical distribution of selenium nanoparticles, the crystalline resulting particles, and the preservation of meat products from which carrageenan-coated burgers were made. These selenium nanoparticles were manufactured using green methods. Therefore, it was demonstrated that dried bitter orange peel extract is a reducing agent for rapidly synthesizing nano-sized selenium nanoparticles.

Authors' Contribution: STH provided formal analysis, developed the methodology, managed the project, secured funding, validated results, and wrote the original draft. AMM contributed to data curation, conducted formal analysis, and assisted in developing the methodology.

Competing Interests: The authors declared no conflict of interest.

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