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Gelatine nanoparticles from sole fish and their usage for mediating selenium nanoparticles and producing functional candies

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Abstract

Fish gelatine offers promising benefits for the nutritional and pharmaceutical industries owing to its biocompatibility, functionality, and cost-effectiveness. In the present work, the gelatine was extracted from sole fish (*Solea solea*) skin, and converted into nanoparticles (GeNPs), and utilised as mediators, stabilisers, and carriers for selenium nanoparticles (SeNPs). The structural characteristics of the nanoparticles and nanocomposites were characterised by using Fourier transform infrared spectroscopy (FTIR), electron microscopy, and light scattering. These analyses revealed that SeNPs were effectively and directly synthesised with the aid of GeNPs and ascorbic acid. The GeNPs exhibited spherical and smooth surfaces with a mean diameter of 281.6 nm. Conversely, the SeNPs and their composites displayed particle sizes of 35.7 and 342.8, respectively. FTIR analysis validated the structural groups and molecular interactions. The physical characterisation of fish gelatine extracted from sole fish revealed bloom gel strength of 339 g, viscosity of 14.6 cP, and a melting point of 21.2°C. Additionally, measurements of the proximate composition, colour, and gel clarity indicated that the fish gelatine exhibited favourable characteristics. The nano-gummies prepared using the nanocomposite of Ge-Selenium (NGe/Se) exhibited 72% antioxidant activity. Furthermore, the nano-gummy maintained its appearance and texture with no significant alteration after 45 days of storage. The production of NGe/Se from sole fish and the subsequent manufacturing of nano-gummies resulted in high-nutritional products with enhanced antioxidant properties and superior sensorial qualities.

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Introduction

Biopolymers, derived from living organisms, are often regarded as safe materials with numerous applications in healthcare, pharmaceuticals, and food industries. They are classified into two classes: (1) polysaccharides (*e.g.*, alginate, cellulose, chitosan, dextran, *etc*.), and (2) proteins (*e.g.*, albumin, collagen, gelatine, *etc*.). Both are biodegradable, hydrophilic, and biocompatible (Yasmin *et al*., 2017; Jabeen and Atif, 2024).

Gelatine (Ge) is a high-quality protein obtained from collagen hydrolysis, and extracted from various connective tissues, bones, and skins of animals (Karayannakidis and Zotos, 2016; Ahmad *et al*., 2023). Pork and cattle bones, bovine hide, and pigskin are commonly used as raw materials for Ge and collagen extraction (Martins *et al*., 2018; Purba *et al*., 2023). However, in the mid-1990s, the global beef industry faced a significant health crisis due to the rapid spread of BSE (Bovine Spongiform Encephalopathy) (Aidat *et al*., 2023). The preparation

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of cattle bones also involves a complex and expensive procedure, requiring demineralisation and degreasing of the raw material to produce ossein, resulting in higher costs compared to fish Ge (Boran and Regenstein, 2010).

Furthermore, Islam prohibits the use of any part related to pork, thus posing challenges regarding the source of Ge for the production of halal foods and goods. Therefore, food scientists have a responsibility to explore safer alternative sources of Ge, such as fish Ge, which is relatively abundant and simple to be extracted from fish skin samples (Alfaro *et al*., 2015; Dara *et al*., 2020).

Fish Ge serves not only as an alternative to mammalian Ge, but also eliminates the risk of viral contamination and BSE (Sila *et al*., 2017), while adding economic value to fish by-products and reducing waste in the seafood sector (Nitsuwat *et al*., 2021). Ge is recognised by the USFDA (United States Food and Drug Administration) as a GRAS (generally regarded as safe) substance due to its long history of safe use in medications, cosmetics, and food products (Sahoo *et al*., 2015; Naghdi *et al.*, 2024). Ge is well-suited for various manufacturing techniques and applications, such as food, photography, medicine, and pharmaceuticals, owing to its numerous benefits, including low cost, wide availability, low antigenicity, ease of use, excellent film-forming, gelling, foaming, and emulsifying properties, as well as biocompatibility and biodegradability (Rafael *et al*., 2021; Yang *et al*., 2021).

Ge is utilised in food products as a film-forming and gelling agent, imparting texture and shape to food items (Yasmin *et al*., 2017; Ahmad *et al*., 2023). The primary objective of Ge production is to convert insoluble collagen into soluble Ge, while maximising yield and maintaining excellent functional properties (Boran and Regenstein, 2010; Naghdi *et al.*, 2024). Collagen undergoes substantial thermal denaturation in acidic or alkaline environments, resulting in denatured protein (Niu *et al*., 2013; Carvalho *et al*., 2018). The production of Ge from raw materials involves five basic phases: washing, extraction, purification, concentration, and drying (Aidat *et al*., 2023; Naghdi *et al.*, 2024).

Fish waste has emerged as a reliable source of halal Ge, meeting various socio-cultural expectations. Waste materials, including scales, bones, skin, head, and viscera, constitute useful bioactive compounds with biological activities and nutrients in fish by-products, ranging from 5 to 18% of the fish weight (Coppola *et al*., 2021; Naghdi *et al.*, 2024). These wastes are utilised in the cosmetics, pharmaceutical, and nutraceutical industries (Karayannakidis and Zotos, 2016; Munawaroh *et al*., 2023). Ge can be extracted from various freshwater and marine sources, including sea mammals, marine invertebrates, and fish.

Sole fish (*Solea aegyptiaca*) is a marine flatfish in the Soleidae family, and its skin represents a significant amount of marine waste by fish processing industries. It offers potential for eco-friendly collagen extraction, leading to effective waste management and increased profitability. Fish skin, rich in collagen, can serve as an excellent substitute for mammalian Ge (Arumugam *et al*., 2018; Munawaroh *et al*., 2023). Additionally, the consumption of fish is highly beneficial to human health (Altintzoglou and Heide, 2016; El-Seedi *et al*., 2023).

Ge is an attractive biodegradable material for application in nanobiotechnology and nanopharmaceuticals (Carvalho *et al*., 2018). Nanotechnology has revolutionised modern scientific domains, particularly in food production, processing, and storage. Its application in the food sector enhances shelf-life stability and strength, and alters food properties such as colour, taste, texture, and other sensory characteristics (Ahmad *et al*., 2023; Singh *et al*., 2023). Nanotechnology enables the nano-encapsulation of bioactive compounds such as probiotics, minerals, antioxidants, antimicrobials, vitamins, biopeptides, enzymes, polyphenols, and targeted drugs, thereby improving their transport capabilities, solubility, and bioavailability to enhance therapeutic effects, and reduce the side effects of formulated drugs (Sahoo *et al*., 2015; Pal *et al*., 2019; El-Seedi *et al*., 2023). Nanoparticles have garnered significant interest in drug delivery research due to their unique characteristics, including smaller particle size, higher drug loading, adjustable shape, modifiable surfaces, non-carcinogenicity, enhanced encapsulation, easy availability, and controlled and targeted release (Khramtsov *et al*., 2021).

Selenium (Se), a micronutrient metalloid, is a crucial component of selenoenzymes, which prevents oxidative damage to animal cells (Ahmadi *et al*., 2021). SeNPs find numerous applications in medicine and nanobiotechnology due to their bioavailability, physicochemical stability, absorption capacity, biocompatibility, and low toxicity (Abdelhamid

et al., 2024). SeNPs serve as photocatalysts, antioxidants, antimicrobials, chemopreventives, and therapeutic agents. They have a significant impact on boosting the immune system and protecting against metal toxicity (Kazemi *et al*., 2020). Nanotechnology improves material quality, structures, and applications across various fields, including industrial catalysis, medicine, and agriculture. Hence, the integration of nanotechnology and green chemistry is essential (Vijayaram *et al*., 2024). Green chemistry advocates for the removal or minimisation of toxic and hazardous components from reactions as much as possible. The development of clean technology necessitates the use of eco-friendly materials. SeNPs are synthesised by reducing Se ions using a reducing agent (vitamin C - ascorbic acid) in the presence of a stabiliser (Kazemi *et al*., 2019; Vijayaram *et al*., 2024).

Many drug delivery methods have been established over the last few decades. Jelly candies, consisting of gelling agents, are one of the most commonly used drug delivery strategies. This approach is widely accepted, especially in children, due to their preferred structure and taste, and these formulations are extensively used nowadays (Hosseini *et al*., 2022). Ge is commonly utilised in gummy candies because of its special functional characteristics (Salama and Hashim, 2022). Stevia is used as a natural sweetener due to its high intensity and stability (Rivero *et al*., 2021). The food industry and health-conscious consumers are always seeking alternatives for producing candies while still maintaining the product's texture, volume, flavour, and shelf life. According to Kurt *et al*. (2022), soft candies can meet customer preferences for healthier sweet items rather than those containing artificial flavouring and colouring agents.

The aim of the present work was to explore the potentiality of utilising aquatic waste for Ge extraction as an eco-friendly waste management strategy that could offer greater profitability. Specifically, the present work focused on formulating and analysing the physical and chemical attributes of Ge extracted from sole fish skin. To advance cleaner technologies, the integration of eco-friendly materials, like GeNPs, was essential. These nanoparticles served as mediators, stabilisers, and carriers for SeNPs, leading to the production of nano-gummies with enhanced nutritional value and heightened antioxidant and sensory characteristics.

Methods and materials

Extraction of fish gelatine

Fish Ge was extracted following the method outlined by Niu *et al*. (2013) with slight modifications. Initially, common sole fish were obtained from the Mediterranean Sea, near Abu Qir Bay, Alexandria, Egypt, kept in ice boxes, and delivered under cooling to the laboratory within 3 h after fishing. Next, 30 g of sole fish skin were soaked in tap water for 10 min to remove contaminants, maintaining a skin-to-water ratio of 1:6 (w/v). Subsequently, the skin was filtered and washed twice to eliminate suspended residues, with excess water removed by manual squeezing. The skin was then immersed in a 0.3 M solution of sodium hydroxide (NaOH) for 1 h (1:6, w/v), followed by filtration and five additional washes. Following this step, the skin underwent a 1-h immersion in a solution of acetic acid (0.18 M) (1:6, w/v), followed by filtration and washing. After the alkaline and acidic treatments, the skin was soaked in a water bath at 45°C for 3 h (1:4, w/v). Various separation techniques, including filtration, drying, and grinding, were employed to isolate the extracted fish Ge. Finally, the sole fish skin was stored at -5°C for future use. The extracted fish Ge yield was calculated using Eq. 1 (Faihana and Abdullah, 2022):

$$Yield\ of\ gelatine\ \% = \frac{Dry\ weight\ of\ gelatine\ (g)}{Wet\ weight\ of\ skin\ (g)} \times 100 \quad \text{(Eq. 1)}$$

Preparation of fish gelatine nanoparticles

Fish GeNPs were synthesised using a two-step dissolvation method with minor adjustments. The initial step involved fractionating LMW (low molecular weight) and HMW (high molecular weight). This process commenced by dissolving 1.25 g of fish Ge in 25 mL of distilled water, and consistently heating it to 40°C to facilitate the fish Ge dissolvation and rapid sedimentation. Subsequently, 25 mL of acetone (the desolvating agent) was introduced to the fish Ge solution to precipitate HMW fish Ge, while the supernatant containing LMW fish Ge was discarded. The HMW fish Ge was then re-dissolved in 25 mL of water and stirred at 720 *g* with constant heating. The second step involved the precipitation phase for nanoparticles (NPs) synthesis, which commenced by adjusting the pH of the fish Ge solution to 2.5, crucial for generating the smallest fish

GeNPs. Following this, 75 mL of acetone was gradually added drop by drop without heating. A glutaraldehyde solution (250 µL) with a 25% concentration (w/v) was incorporated as a crosslinking agent towards the end of the procedure. The solution was then stirred at 720 *g* for 12 h. Subsequently, the solution underwent centrifugation (using a SIGMA 2-16KL centrifuge; Sigma Lab. GmbH, Germany), and was re-dispersed in 30% acetone to purify the particles. Finally, the NPs were stored at 2 - 8°C (Jahanshahi *et al*., 2008; Subara *et al*., 2018).

Preparation of nanocomposite from Ge-selenium nanoparticles (NGe/Se)

First, a solution of 10 mg/L Na_2SeO_3 (sodium selenite; molar mass: 172.94 g/mol; Sigma-Aldrich, MO) in deionised water (DIW) was prepared as the selenium source. Equal volumes of the Na_2SeO_3 solution and GeNPs (10%), acting as a stabilising agent (20 mL each), were combined and stirred at 720 *g* using a magnetic stirrer. Ascorbic acid (0.25%) was then added dropwise as a reducing agent at a standard temperature of 80°C until the appearance of an orange-to-reddish colour indicated the synthesis of NGe/Se (Kazemi *et al.*, 2019; 2020).

Characterisation

FTIR (Fourier-transform infrared spectroscopy)

One of the most effective spectroscopic methods for food analysis is infrared (IR) spectroscopy, which offers insights into the functional groups, chemical structures, morphology, physico-chemical properties, biochemical bonds, interactions, and intermolecular crosslinking of foods and bio-substances (Riaz *et al*., 2018). Fish Ge, GeNPs, and NGe/Se were analysed using FTIR (JASCO FT-IR-360, Tokyo, Japan). The FTIR absorption spectra were observed within the range of 4,000 to 500 cm^{-1} using the FTIR spectrophotometer.

NPs' ultrastructure

The external morphology of GeNPs and NGe/Se was observed using SEM (JSM IT100, JEOL, Tokyo, Japan), operating at a 20 kV accelerating voltage. TEM (JEOL JEM-2100, JEOL Ltd., Tokyo, Japan) was employed to characterise the size, shape, and morphology of NGe/Se. Before application to a carbon-coated copper grid, the sample was diluted with deionised water, and the excess sample was removed. Imaging was performed following complete drying of the grid at room temperature.

Zeta potential and particle size distribution

A Zetasizer (Malvern Nano ZS instrument, Southborough, MA) was employed to assess the zeta potential and particle size distribution of the synthesised NPs using dynamic light scattering analysis (DLS).

Physical characteristics of sole fish skin gelatine

Gel strength of sole fish skin gelatine

One of the most crucial quality factors utilised in the Ge industry for distinguishing between different gelatines is bloom strength. To prepare a fish Ge solution (6.67%, w/v), 7.5 g of fish Ge was dissolved in 105 mL of distilled water in standard bloom bottles. The solution was hydrated at 25°C for 1 h, followed by incubation at 45°C with periodic stirring until complete dissolution. The solution was then cooled to 25 ± 2°C for 30 min before gelation at 4°C in a refrigerator for 18 h. Gel strength was evaluated using a texture analyser (Li *et al*., 2019).

Viscosity of sole fish skin gelatine

The viscosity (measured in cP) of a 10 mL solution of fish Ge was assessed using a Brookfield digital viscometer (Model DV-E, Brookfield Engineering USA) fitted with a No. 1 spindle at a temperature of 45°C (Cho *et al*., 2006).

pH of sole fish skin gelatine

The pH of the fish Ge solution was determined following the standard test procedure of BSI 757:1975. A fish Ge solution was prepared in a bloom bottle by adding 1 g of fish Ge to 100 mL of distilled water at 60°C. The pH meter (CyberScan 100; Thermo Fisher Scientific, IL) was used to measure the pH of the solution at 45°C (Songchotikunpan *et al*., 2008).

Melting point of sole fish skin gelatine

Test tubes with screw caps were filled with a solution of fish Ge (6.67 g/100 mL of distilled water). The samples were stored in a refrigerator at 5°C for 16 - 18 h, and then inverted into a 10°C water bath so that the headspace was at the base. The water bath was warmed at a rate of 1°C per minute, and the melting temperature of the gel was measured as gas moved into the headspace (Pranoto *et al*., 2007).

Proximate composition of sole fish skin gelatine

The moisture content of fish Ge was evaluated by oven drying (E 28; BINDER GmbH, Tuttlingen, Germany), and the fish Ge was extracted at 105°C for 24 h. The total lipids were determined gravimetrically following a Soxhlet extraction with hexane. The ash content was evaluated using a muffle furnace at 550°C for 24 h. The total content of crude proteins was calculated using a Kjeltec 8400 analyser (FOSS, Hilleroed, Denmark) and the Kjeldahl technique. A conversion factor of 5.5 was used for nitrogen content to protein conversion (Mi *et al*., 2019).

Colour of sole fish skin gelatine

A Minolta CR-400 Colorimeter (KONICA MINOLTA, INC., Tokyo, Japan) was utilised to determine the colour of fish Ge powder following the CIE system parameters: L* (lightness or brightness), a* (redness), and b* (yellowness) (Mi *et al*., 2019).

Preparation of jelly candies

Following the described methods (de Avelar and Efraim, 2020; Rivero *et al*., 2021) with slight modifications, the sugar solution [containing stevia (10%, w/v) and sucrose (22%, w/v)] was amended with NGe/Se containing solution (10%, w/v); the solutions were separately prepared and dissolved at 75°C under stirring, then equal amounts from both solutions were mixed and stirred. Subsequently, the two solutions were mixed for 30 min at 50°C to obtain the main gelling solution. The pH value of all solutions was maintained at 3, and adjusted with the addition of 1.2 M of citric acid solution. Finally, the solution was poured into silicon moulds, and cooled in a refrigerator at 4°C for 24 h (Cebi *et al.*, 2019).

Antioxidant activity of nano-gummies

The antioxidant activity was assessed using the DPPH assay (2,2-diphenyl-2-picrylhydrazyl hydrate). Briefly, the sample was mixed with DPPH, which is deep violet in methanol solution and turns colourless when combined with a substance that donates hydrogen atoms as an antioxidant (Vera *et al*., 2016; Ahmadi *et al*., 2021). The mixture was then incubated for 30 min in the dark. UV/Vis spectrophotometry (model UV-2450, Shimadzu, Japan) was used to measure the absorbance of the final combination at 517 nm (Boroumand *et al*., 2019; Sulaiman *et al*., 2022). The antioxidant activity was calculated using Eq. 2 (Ali *et al*., 2020):

$$\text{Inhibition percentage of DPPH} = [(A0 - A1) / A0] \times 100 \quad \text{(Eq. 2)}$$

where, A0 = control's absorbance value, and A1 = test sample's absorbance value.

Sensory evaluation of nano-gummies

Sensory evaluation is a scientific technique used for analysing and measuring responses to a product by documenting the assessor's replies and comments based on aroma, taste, appearance, texture, and overall liking. A questionnaire and sensory evaluations were created based on a five-grade scale. Healthy volunteers of neutral gender, aged 18 to 45, with no sensitivities or medical histories related to the components used in the production of gummies, were eligible to participate. Participants under the age of 18, smokers, those suffering from flu or other similar symptoms, and individuals sensitive to the components used in making gummy candy, were excluded. The categories for these sensory grades were texture, aroma, appearance, colour, overall quality, acceptability, and taste. The maximum sensory value demonstrated the best performance in this test.

Statistical analysis

Data were subjected to One-way ANOVA and Student *t*-test analyses, where $n = 3$, with results presented as mean ± SD (standard deviation), utilising Microsoft Excel and GraphPad Prism 8.0.2 software. Statistical significance was determined at a p value of < 0.05.

Results and discussion

Extraction of fish gelatine

Fish Ge was effectively extracted from sole fish skin using both alkali and acid treatments, as well as the hot water extraction method (Figure 1). The yield (%) of the extracted sole fish skin Ge was 8% at 45°C (Eq. 1).

The sole fish skin can be utilised as a source of fish Ge extraction due to its high yield, protein content, gel strength, and viscosity. Therefore, it has the potential to be used as an alternative source of Ge in the food industry (Sawant *et al*., 2016). In fish Ge extraction, using both alkali and acid treatments results in higher yields of fish Ge with improved quality compared to using a single treatment

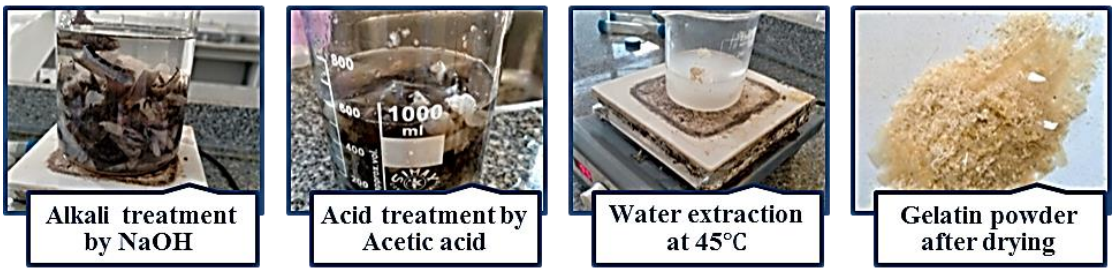

Figure 1. Steps of fish gelatine extraction from sole fish skin.

technique (Ranasinghe *et al*., 2022). The widely used method for Ge extraction is the hot water extraction method, which breaks down the structure of Ge, and is influenced by the time and temperature of extraction (Ranasinghe *et al*., 2022). Previous studies indicated that NaOH solutions had better ability to eliminate non-collagenous proteins, while acetic acid had swelling effect on fish skin (Viji *et al*., 2019; Ahmad *et al*., 2023). Swelling is crucial as it results in the unfolding of proteins by breaking the non-covalent bonds, exposing collagen to solubilisation and extraction (See *et al*., 2015).

Preparation of gelatine nanoparticles (GeNPs)

GeNPs were successfully synthesised using the two-step dissolving process. Acetone, serving as a desolvating agent, facilitated the precipitation of HMW fish Ge, while the LMW fraction remained in solution. Adjusting the pH to 2.5 during the second step was crucial for generating the smallest GeNPs. The gradual addition of acetone, followed by the introduction of glutaraldehyde as a cross-linking agent, contributed to the formation of stable nanoparticles. Synthesising nanoparticles from Ge at room temperature (25°C) was impractical due to the formation of a highly viscous gel. Heating the solution to 40°C allowed for sufficient uncoiling of the protein chains, reducing solution viscosity, and facilitating dissolution. The size of the GeNPs was observed to increase with higher pH, stirring time, and acetone concentration, while decreasing with higher glutaraldehyde volume and stirring speed (Sahoo *et al*., 2015).

Acetone acted as a co-solvent in GeNPs synthesis, with higher concentrations resulting in larger GeNPs. Its high miscibility in water prevented denaturation of Ge, ensuring the stability and spherical shape of the GeNPs (Sahoo *et al*., 2015). Glutaraldehyde, a non-toxic compound, was effective as a crosslinking agent, contributing to particle stability. The size of the GeNPs decreased with higher glutaraldehyde volume and stirring speed as the energy required for particle shearing increased. It was also reported elsewhere that increasing the stirring speed from 180 to 720 *g* resulted in a decrease in the size of GeNPs (Subara *et al*., 2018).

Interestingly, pH lower than 2.5 did not lead to a visible increase in particle size, contrary to previous findings with mammalian Ge. This discrepancy suggested differences in Ge behaviour between species, and underscores the importance of optimising synthesis conditions for specific applications. Previous studies have indicated that the optimal pH for creating the smallest porcine GeNPs was 3.25, highlighting the need for species-specific optimisation in nanoparticle synthesis (Subara *et al*., 2018).

Preparation of nanocomposite of Ge-Selenium nanoparticles (NGe/Se)

The effectiveness of the method in generating NGe/Se was exemplified by the successful synthesis achieved. The addition of Na_2SeO_3 and GeNPs facilitated the incorporation of selenium into the Ge matrix, with ascorbic acid serving as a reducing agent to promote the reaction. The orange-to-reddish colour observed upon addition of ascorbic acid indicated the formation of NGe/Se. This method offers a simple and efficient approach to producing NGe/Se for various biomedical and industrial applications. These colloidal solutions maintained aggregation stability for months, and did not substantially scatter light (Figure 2).

FTIR

The FTIR analysis was utilised to identify the substances based on their functional groups in order to compare the chemical structures of Ge, GeNPs, and NGe/Se (Figure 3) (Subara *et al*., 2018). All samples exhibited different absorption zones for different

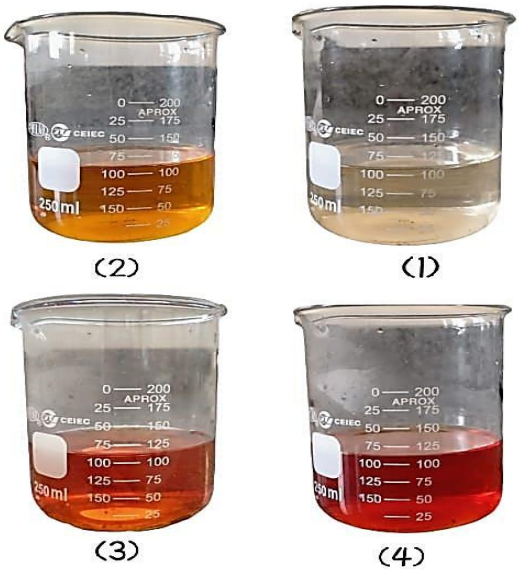

Figure 2. Preparation of nanocomposite gelatine-selenium (NGe/Se); **(1)** NGe/Se composite at 0; **(2)** NGe/Se composite after 30 min; **(3)** NGe/Se composite after 1 h; and **(4)** NGe/Se composite after 2 h.

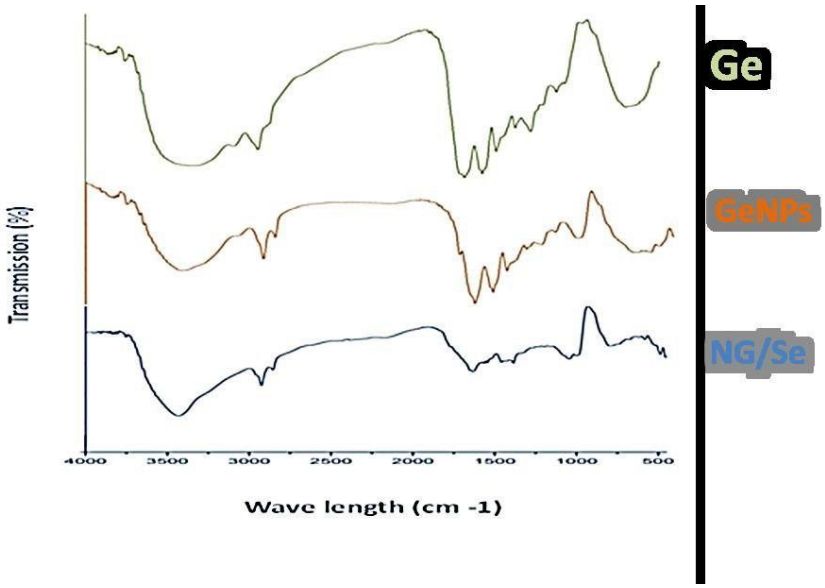

Figure 3. FTIR spectra of fish gelatine (Ge), gelatine nanoparticles (GeNPs), and nanocomposite gelatine-selenium (NGe/Se).

amides (amide A, B, I, II, and III). The FTIR spectrum was characterised by a shift in the absorption area of amides (A, B, I, II, and III) (Muyonga *et al*., 2004).

FTIR spectra of fish gelatine

Amide A shows NH group vibration due to the presence of hydrogen bonds and OH group vibration. It is observed as a broad band around 3350 cm^{-1}. Amide B indicates an unsymmetrical stretching vibration of the $-NH_3$ and =C-H groups, with a characteristic peak at 2925 cm^{-1} in the present work. The presence of the amide A and B regions indicated the existence of functional groups (CH, OH, and NH) in the fish Ge samples, which are typically located in the spectral region between wave numbers 4000 - 2500 cm^{-1}. Amide I signifies the presence of the OH group combined with COO– or a C=O stretching vibration. Within the wave numbers of 1650 - 1550, fish Ge exhibited functional groups such as C=N, C=C, and N=O. Ge extracted from sole fish showed amide I regions at 1550, 1600, 1625, and 1650 cm^{-1}. The amide II absorption band in fish Ge typically occurs around 1560-1335 cm^{-1}. In the present work, the amide II region was observed at 1350, 1375, and 1400 cm^{-1}. This region arises due to the vibration of

C=N stretching, and the distortion of the NH bond in the protein. Amide III is located in the region of 1300 - 1000 cm^{-1}. In the present work, amide III peaks were observed at 1050, 1075, 1125, 1175, 1200, 1250, and 1275 cm^{-1}. Amide III typically exhibits a mixture of peaks between vibrations of the NH group and the CN group strain. The presence of a short NH group peptide indicates molecule degradation during the fish Ge extraction procedure (Hidayati *et al*., 2021).

FTIR spectra of fish gelatine nanoparticles

In GeNPs, a characteristic band was noticed at 3425 cm^{-1} because of the N-H stretching of the amine group, and amide A appeared at 2925 cm^{-1} as a result of the stretching vibration of C–H bonds. Amide B was found at 1650 cm^{-1} which refers to the C=O stretching of the amide group. Amide I appeared at 1550 cm^{-1} due to C-N stretching vibration. Amide II was found at 1450 cm^{-1}. At 1450 cm^{-1}, a stronger peak could be related to the aldimine linkage (CH=N), and was generated by the reaction of the amino group of fish Ge with the aldehyde group (-CHO) of the cross-linking agent (glutaraldehyde) (Subara *et al*., 2018; Viji *et al*., 2019). Amide III appeared at 1175 cm^{-1}, which showed some peaks between NH group and CN group vibrations. The NH group explains why the molecule degraded at the time of fish Ge extraction. The typical absorption peaks of fish Ge C-H bonds appeared at 2900 cm^{-1}, corresponding to forms that are symmetric and asymmetric. Hydrogen bonds with amine groups, carboxyl, and –OH were observed at about 3400 cm^{-1}. The frequency of the C–H stretching vibration led to the appearance of a weak absorption peak at 2200 cm^{-1}.

FTIR spectra of nanocomposite Ge-selenium

A very strong peak at 1650 cm^{-1} was observed to disappear, while some peaks appeared below 1500 cm^{-1}, which is attributed to bending vibration modes of the C-C and C–H bands (Kazemi *et al*., 2020). These results indicated the creation of new bonds, and we can conclude that GeNPs adhered successfully to the surface of SeNPs (Figure 3).

NPs' ultrastructure

The physical characteristics of the synthesised NPs were assessed through electron microscope imaging (Figure 4). TEM analysis revealed that SeNPs exhibited a uniform size distribution and excellent dispersion. SEM imaging confirmed the formation of spherical and smooth NPs without any observed heterogeneity or hairline cracks. The mean diameter of GeNPs, SeNPs, and NGe/Se was estimated to be 281.6, 35.7, and 342.8 nm, respectively.

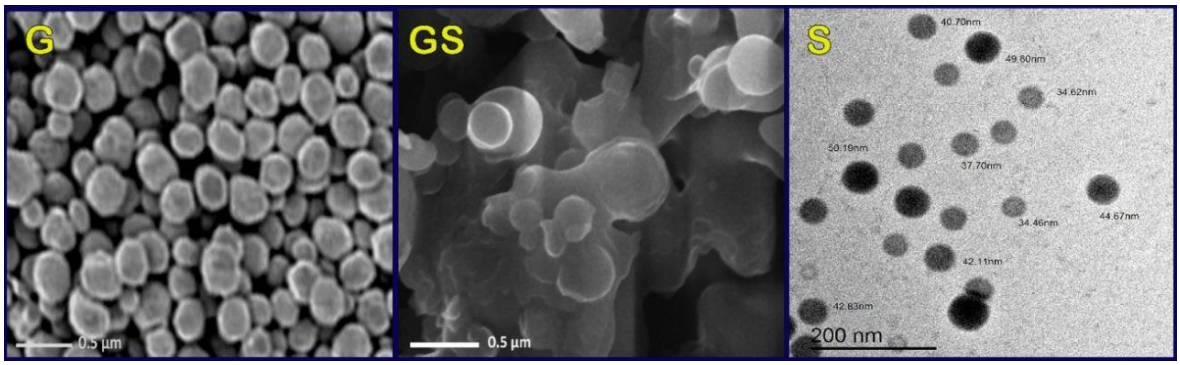

Figure 4. Ultrastructure of synthesised nanoparticles; **(G)** scanning image of gelatine nanoparticles, **(GS)** scanning image of nanocomposite gelatine selenium, and **(S)** transmission image of selenium nanoparticles.

Zeta potential and particle size distribution

Dynamic light scattering (DLS) analysis conducted using a Zetasizer confirmed the findings. The mean diameters of the particles measured for GeNPs, SeNPs, and NGe/Se were 281.6, 35.7, and 342.8 nm, respectively (Table 1).

Furthermore, the zeta potential measurements revealed that GeNPs exhibited negative charges (-24.23 mV), which slightly decreased to -30.81 mV upon combination with the selenium solution. In contrast, SeNPs displayed a negative charge of -36.79 mV (Table 1).

Physical characteristics of sole fish skin Ge

The bloom gel strength, which is the most significant physical characteristic of fish Ge, was about 339 g. Gel strength depends on the amino acid composition, the size of protein chains, concentration,

Table 1. Size and charge of nanoparticles (GeNPs, SeNPs, and NGe/Se composite).

Nanomaterial	Diameter range	Mean diameter (nm)	Zeta potential (mV)
GeNPs	103.4 - 468.2	281.6	-24.23
SeNPs	26.3 - 57.4	35.7	-36.79
(NGe/Se)	124.6 - 719.1	342.8	-30.81

pH, temperature, presence of additives, molecular weight distribution, and the hydrogen bonds formed by gelatine's free amino acid hydroxyl groups and water molecules (Arnesen *et al*., 2002; Bhat *et al*., 2009; Sawant *et al*., 2018). The viscosity, regarded as the second-most essential physical characteristic of Ge, was 14.6 cP. The pH of the extracted fish Ge at 45°C was 4.51, and the melting point of the extracted gelatine was 21.2°C (Table 2).

Table 2. Functional characteristics of the extracted sole fish skin gelatine.

Functional characteristic	Value
Bloom gel strength	339 ± 0.09
Viscosity	14.6
pH	4.51 ± 0.5
Melting point	21.2 ± 0.09

Data are mean ± SEM of three replicates.

Proximate composition of sole fish skin Ge

The moisture content of the extracted sole fish Ge at 45°C was 7.9%. The fat content (%) of the extracted sole fish Ge was 1.10%. The ash content (%) of the extracted sole fish Ge, which indicates the mineral composition of Ge, was 1.50%. The protein content (%) of the extracted sole fish skin Ge at 45°C was 89% (Table 3).

Table 3. Proximate composition of sole fish skin gelatine.

Component	Value (%)
Moisture	7.9 ± 0.3
Fat	1.10 ± 0.10
Ash	1.50 ± 0.03
Protein	89 ± 0.04

Data are mean ± SEM of three replicates.

According to Sawant *et al.* (2016), the proximate composition (%) of the extracted fish Ge varied significantly ($p \leq 0.05$) in terms of fat, protein, ash, and moisture. The highest protein content and the lowest fat and ash levels were obtained at 45°C. Moisture content was influenced by the humidity during storage, and the duration of drying. Low moisture content is a barrier to accurately determining the physiochemical characteristics of fish Ge. In the present work, the moisture content of the extracted fish Ge was well below the permitted limit for edible gelatine's moisture level (15%). The ash content was also below the suggested maximum limit of 2.6%, as well as the 2% limit for edible fish Ge, indicating the high quality of fish Ge.

Colour and gel clarity of sole fish skin Ge

The colour of the extracted Ge at 45°C was pearly white, and varied significantly ($p \leq 0.05$) in terms of lightness (L*), redness (a*), and yellowness (b*). The lightness value was 90 ± 0.05, the redness value was 1.84 ± 0.03, the yellowness value was 2.90 ±0.05, and the transmittance (%) at 45°C was 80 ±0.02% (Table 4).

Table 4. Colour and gel clarity of sole fish skin gelatine.

Gelatine colour and gel clarity	Value
Lightness (L*)	90 ± 0.03
Redness (a*)	1.84 ± 0.03
Yellowness (b*)	2.90 ± 0.05
Transmittance (%)	80 ± 0.02

Data are mean ± SEM of three replicates.

The colour and clarity of Ge gels are crucial functional characteristics for commercial use. Customers typically prefer lighter colours over darker ones because they associate purity with a lack of colour. Turbidity and dark colour in Ge are often

caused by inorganic substances, proteins, and impurities released during its extraction. Aggregates formed from prolonged exposure of proteins to high temperatures also contribute to increased turbidity (Sawant *et al*., 2016).

Preparation of jelly candies

Twenty healthy candies were successfully produced utilising stevia as a natural sweetener, resulting in candies with a noteworthy 72% antioxidant activity. The nanocomposite of NGe/Se, which comprised GeNPs and SeNPs, was used as the gelling agent for candy production. Each candy contained 225 µg of Se as an antioxidant, and 250 ppm of ascorbic acid, serving as a source of vitamin C (Figure 5).

Figure 5. Nano-gummy candies.

Antioxidant activity of nano-gummies

Results revealed that NGe/Se exhibited a higher DPPH radical scavenging activity compared to fish Ge particles. The observable transformation of the DPPH colour from purple to pale yellow indicated a significant scavenging activity of 72% for each candy.

Sensory evaluation of nano-gummies

The present work compared commercial gummies with nano-gummies. Table 5 presents the sensory evaluation data collected on days 1 and 45 after storage. The aroma of the commercial gummies was notably superior to that of the nano-gummies. After 45 days of storage, all gummies had lost their aroma, with the commercial gummy exhibiting the most significant decline. Although the commercial gummy initially had better appearance and texture, it deteriorated more significantly over the 45-day storage compared to the nano-gummy, which maintained its appearance and texture with minimal changes. The commercial gummy had the highest taste rating. The overall preference and acceptance of the nano-gummy were compared to those of the commercial gummy. Results indicated no significant difference between days 1 and 45.

Table 5. Average sensory evaluation results on day 1 and 45.

	Nano-gummy		**Commercial gummy**	
	Day 1	**Day 45**	**Day 1**	**Day 45**
Aroma	5 ± 0.8	4.6 ± 1.1	5.5± 0.7	4.7 ± 0.6
Appearance/texture	6.6 ± 0.8	6.8 ± 1.3	7.5 ± 1.2	7.3 ± 0.9
Taste	6.9 ± 1.5	6.7 ± 1.0	7.3 ± 1.4	7.4 ± 0.7
Overall liking	6.4 ± 0.7	6.3 ± 0.5	7.3 ± 1.0	7.4 ± 1.1

Conclusion

Nanoparticles synthesised *via* eco-friendly methods are gaining importance due to their exceptional stability and potent bioactive properties, making them valuable across various applications. Green-manufactured NGe/Se is particularly significant for diverse medical applications owing to their low toxicity profile. Incorporating these nanoparticles into jelly candy presents an innovative and promising approach for drug delivery, particularly appealing for paediatric use due to its palatability. This approach is exemplified by the production of health-oriented candies employing stevia as a natural sweetener, which also offers 72% antioxidant activity. Each candy piece was fortified with Se (225 µg) for its antioxidant properties, and ascorbic acid (250 ppm) as a source of vitamin C.

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