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Development and Nutritional Evaluation of Egg Shell Powder and its Nanoparticles

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Abstract

Egg shell waste is available in huge quantities from food processing, egg breaking and hatching industries. It is estimated that worldwide about 250,000 tonnes of egg shell waste is produced annually. Development and characterization of egg shell powder would result in additional benefit of substantial and better physical, chemical and biological properties. Thus the aim of the study was to develop and nutritionally evaluate egg shell powder and its nano particles. The egg shell nanoparticles were prepared by the method of chemical precipitation using aqueous slurries of egg shell bio-waste and treatment with phosphoric acid and ultrsonication. The developed nanoparticles were characterized by UV spectra, SEM, zeta potential and FTIR. Results revealed that the developed nano particle size was 79.1nm and spherical in morphology. Zeta potential analysis revealed less stability of egg shell nano particle. FTIR analysis showed significant peak of egg shell particles at 1106cm $^{-1}$ *and two absorbable peaks at 1197cm* $^{-1}$ *and 912cm* $^{-1}$*. Macro and micronutrient of egg shell powder and its nanoparticles were similar. Hence egg shell powder and its nanoparticles may be considered as a natural, novel and potential source of calcium supplementation. In depth studies are recommended to substantiate the results obtained in the study.*

Keywords: *Egg shell, nano particles, characterization, calcium, FTIR, SEM*

Introduction

Poultry is one of the fastest growing segments of the agricultural sector in India, with a poultry population of 489 million, producing 47 billion eggs per year thereby ranks as the third highest among the egg producing countries in the world [1].

Egg shell waste is available in huge quantities from the food processing, egg breaking and hatching industries. About 250,000 tonnes of egg shell waste is produced annually worldwide by the food processing industry only. Uronic acid, sialic acid, alanine and glycine are high in the organic matter of egg shell waste compared to shell membranes [2].

Egg Shell which is discarded has multiple benefits in terms of nutrition and availability. The egg shell is rich in calcium hence it can be used in most of the medical conditions such as osteoporosis. The egg shell can be a novel source of dietary supplement to increase the bone mineral density, especially in post-menopausal women and various other bone deformities. Though few studies are reported in countries other than India, systematic reports on nutrient content, its nano particle preparation and application on various health issues are not available in India. Hence, this study attempts to develop and characterize egg shell powder and its nanoparticles and compare its nutrient content.

Materials and methods

Egg shell waste primarily contains calcium, magnesium carbonate (lime) and protein. In order to maximise the recycling opportunities for egg shells, the material could be incinerated independently of other wastes. The calcium / magnesium content of the shells will be converted into calcium/ magnesium oxide and the resultant burnt lime could be used as a liming agent. The membrane free shell powder can be used in the paper industry or in agriculture as a lime substitute or calcium supplement.

Development of egg shell powder

The egg shell required for the study was collected from the local bakery in fresh form. One kilogram of the egg shell used by the bakery per day was collected of which 500g was used for the development of egg shell powder and 500g of egg shell was used for the development of egg shell nanoparticles.

Development of egg shell nano particles

Method of chemical precipitation was used to prepare nanoparticles of egg shell[3]. Aqueous slurries consisting of egg shell and phosphoric acid solution was used. The egg shells were collected and then cleaned thoroughly. The cleaned egg shells were then powdered with mortar and pestle. The powdered egg shell was washed with 100 ml of acetic acid thrice. Finally, the egg shell powder was washed with water.

The egg shells were placed in oven at 150°C for five hours to remove acid and water, at 500°C for next three hours to remove organic residue and another two hours to convert the egg shell to calcium oxide.

Thermal treatment was followed by transformation of egg shells into hydroxyapatite in phosphate solution. One hundred grams of the calcium oxide was taken from the thermal treated eggshell and was dissolved in 119 g phosphoric acid by consequent addition of 15 g of EDTA and 1600ml of distilled water. The pH of the obtained solution was adjusted to 10 by adding ammonia solution. To enhance nano crystalline growth, the mixture was left undisturbed for 24 hours. After a day, the slurry was centrifuged at a speed of 500rpm for 2 minutes. It was then washed with distilled water. The slurry was re-centrifuged to reach a pH of 7. Finally, it was suspended homogenously in distilled water. With application of mild suction, the sludge was filtered by using Buchner funnel. Taken 100ml of ethanol and filtered precipitate was dispersed in it and subjected to ultrasonification for one hour. The powder was kept in muffle furnace and initially dried at 400°C and subsequent increase in temperature at an interval of 100°C upto 1200°C to obtain the nanoparticles. Five hundred grams of uncrushed egg shell yielded 30 g of nanoparticles.

Characterization of egg shell nanoparticles

Ultraviolet-visible spectroscopy analysis

Ultraviolet-visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The synthesized egg shell nano particle was initially characterized with UV-visible spectrophotometer. Absorption measurements were carried out on UV - specord 210 plus at a resolution of 1nm. UV visible analysis ranges from 200 to 600 nm.

Particle size analysis

Particles are 3-dimensional objects and unless they are perfect spheres (e.g. emulsions or bubbles), they cannot be fully described by a single dimension such as a radius or diameter. The particle size analysis was performed in SZ-100 nano particle Size Analyzer. The instrument is Dynamic Light Scattering (DLS) type with unique size detectors at 173 and 90 degrees. The light sources were DPSS 532 nm, 10 mW laser. The particle size of 0.3 to 8000 nm may be detected with a dynamic range 26,667:1.

Scanning Electron Microscopic (SEM) analysis

The surface topography of nanoparticles was observed with a Scanning Electron Microscope (SEM). These were characterized using Jeol Model - 6390 Scanning Electron Microscopy (SEM). The image mode of the microscopy is secondary electron image, detected by the E. T detector. The electron gun used in the microscopic analysis accelerates at voltage range of 0.5 - 30 KV and the filament is pre-centered tungsten hairpin filament.

Zeta potential analysis

Zeta potential is the charge on a particle at the shear plane. This value of surface charge is useful for understanding and predicting interactions between particles in suspension. The zeta potential analysis was performed in SZ-100 nano particle size analyzer.

The measurements were made by adding a small amount of suspension or emulsion to the measurement cell and inserting the cell into the instrument. The instrument software then automatically determines the appropriate electric field strength, adjusts the reference beam intensity to ensure the optimal signal to noise ratio, collects and analyzes the data and presents the results to the user. Often, the effect of H^+ or other ions on zeta potential is important. In the former case, a pH titration can be performed, and in the latter, the ion concentration is varied (usually on a logarithmic scale) and a series of zeta potential measurements are performed. Significant labor savings can be realized by using an automated titrator to adjust sample pH.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The spectrum in FITR represents the molecular absorption and transmission, creating a molecular finger prints of the sample. Fourier Transform InfraRed Spectroscopy (FTIR) spectrum of samples was recorded on ATR-FTIR brucker alpha (transmission) instrument with a diffuse refluctance mode (DRS - 8000) attachment. All measurements were carried out in the range of 600 to 6000 cm^{-1} at a resolution of $6cm^{-1}$.

Evaluation of nutrient content

Moisture

Weighed definite amounts of food material (2g) in each dish and placed in an electric oven thermostatically controlled at 100-105°C. Heated for a stipulated time (2 hours), cooled in a desiccator for half an hour and weighed. This was also weighed till successive weighing showed no further loss:

Macronutrient

Carbohydrate was analysed by anthrone method, fat by soxhlet extraction and protein by kjeldahl analysis using standard procedures

Micronutrients

Calcium and iron contents were analysed by standard procedures4. Minerals namely magnesium, zinc and copper were analyzed using the solution obtained by dry ashing the samples at 550°C. The ash was dissolved in 10 per cent HCl (25 ml) and 5 per cent lanthanum chloride (2 ml), heated to boiling, filtered into 50 ml standard flask and made up to volume with distilled deionised water. The minerals were determined in an atomic absorption spectrophotometer. For the analysis of selenium, sample materials were digested with nitric acid- perchloric acid mixture. 0.5 g of sample was digested with 12 ml of 5:1 conc HNO_3 - perchloric acid mixture till the organic carbon is completely removed. Volume of the extract was made up to 5 ml with 1 N HNO_3. The sample solutions were then kept in refrigerator to avoid evaporation. The metal concentration was determined by Atomic Absorption Spectrophotometry involving direct aspiration of the solution using graphite furnace. Absorption of selenium was read at 196 nm with a slit width of 0.2nm[4].

Results and Discussion

The egg shells were crushed and then blended to make powder. A fine and white powder of egg shell was obtained. Clinical and experimental studies showed that egg shell powder has positive effects on bone and cartilage and that it is suitable in the prevention and treatment of osteoporosis. The egg shell powder was used as a stabilizing material for improving soil properties[5], as a source of calcium for piglets[6] and as a source of calcium in human nutrition[7].

Dutch researchers have reported recently a highly positive effect of egg shell calcium (with added magnesium and vitamin D) on bone mineral density in a scientific study (double blind, placebo-controlled) [8].

Characterization of egg shell nanoparticles

The results of characterization using a variety of different techniques are presented below.

UV Visible spectroscopy analysis

The principle of UV visible spectroscopy is that the molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons, the longer the wavelength (Figure 1) of light it can absorb.

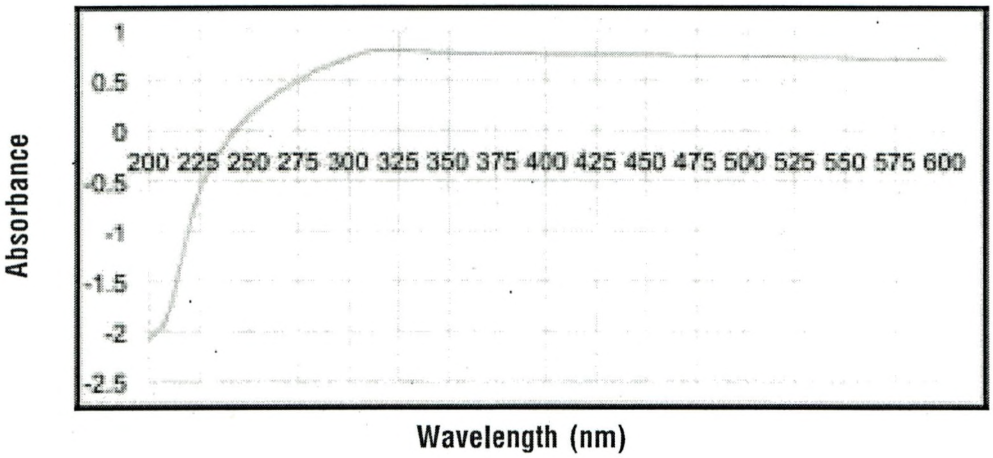

Figure 1
UV visible spectra of egg shell nanoparticles

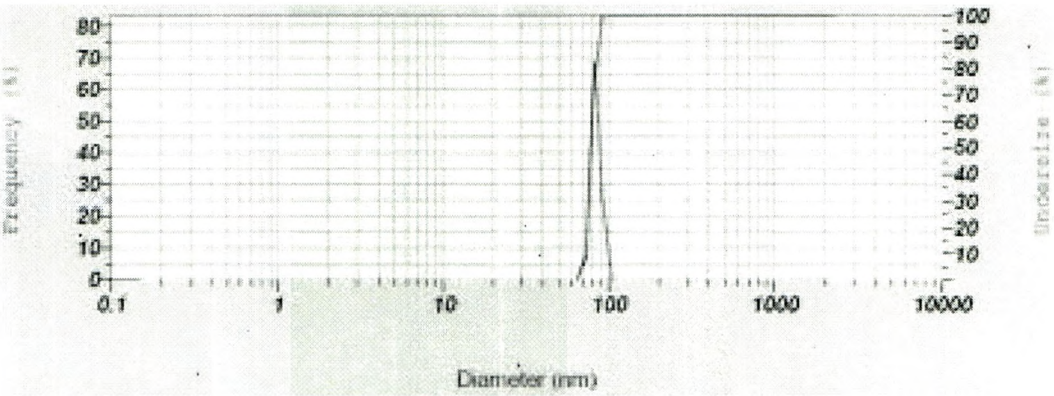

Figure 2
Particle size analysis of egg shell nanoparticles

The graph represents the results of UV visible spectroscopy analysis on egg shell nanoparticles. The results showed a peak at 320 nm wave lengths and then the absorbance sustained at the same level.

Particle size analysis

In order to simplify the measurement process, it is often convenient to define the particle size using the concept of equivalent spheres.

Figure 2 shows the particle size results of the nano egg shell particles. The mean, standard deviation and mode of nano egg shell particles are 79.1nm, 5.2nm and 78.5nm respectively, revealing that the egg shell nano particle has good surface area and absorbance.

Scanning Electron Microscope (SEM) analysis

A Scanning Electron Microscope (SEM) is a type of electron microscope that images a sample by scanning it with a higher energy beam of electrons in a faster scan pattern. The SEM image shows the development of egg shell nanoparticles.

The SEM micrographs (Figure 3) of nano particle obtained in the filtrate showed that they are round in shape and randomly dispersed on the surface. The size of the particle was small and found to be in the range of 35nm to 50nm. This confirmed the development of nanoparticles of egg shell powder.

Zeta potential analysis

Zeta potential is the charge on a particle at the shear plane. This value of surface charge is useful for understanding and predicting interactions between particles in suspension. Manipulating zeta potential is a method of enhancing suspension stability for formulation work

The zeta potential analysis proves the stability of the nano particles.

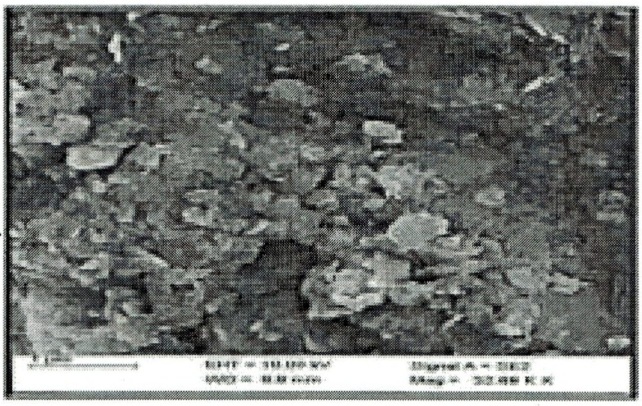

Figure 3
SEM image of egg shell nanoparticles

Figure 4 reveals that the eggshell nano particles is less in stability as the value is obtained in negative range. The normal stability of the nanoparticle ranges from -30mV to +30mV.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The results of the ultimate analysis of the Fourier Transform Infra Red Spectroscopy (FTIR) is shown in Figure 5. The most significant peak of intensity of nano egg shell particles at $1106 cm^{-1}$, strongly associated with the presence of carbonate minerals within the egg shell matrix. There are also two observable peaks at about $1197 cm^{-1}$ and $912 cm^{-1}$, respectively, which may be associated with the presence of calcium carbonate.

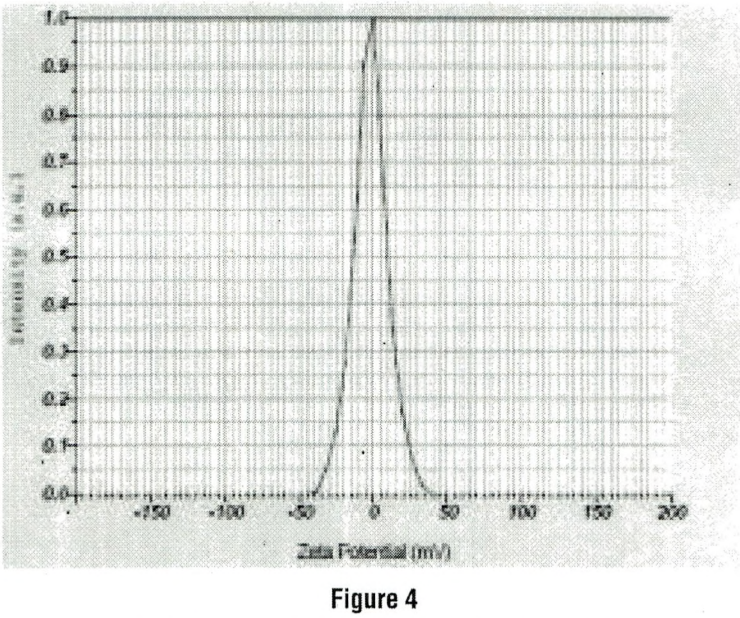

Figure 4
Zeta potential analysis of egg shell nanoparticles

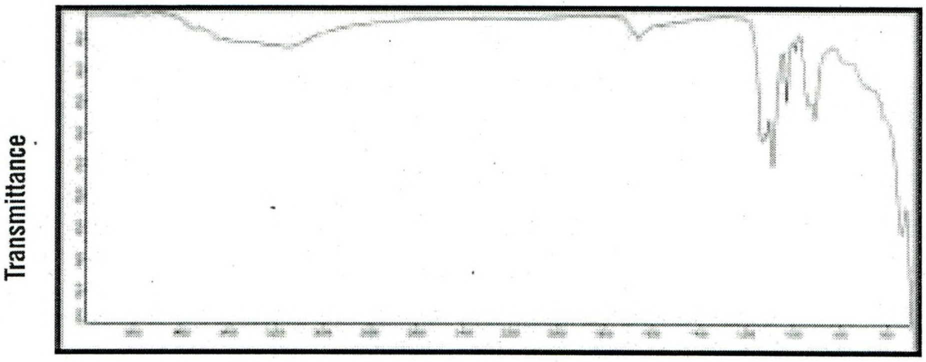

Figure 5
FTIR spectra of egg shell nanoparticles

Macronutrient content of egg shell powder and its nanoparticles

Table I shows the macronutrient composition of egg shell powder and its nanoparticles.

The egg shell powder contains 0.349g per 100g of moisture and the nano egg shell powder contains 0.26g per 100g. The moisture level of the egg shell and nano egg shell powder indicates that the bacterial growth will be low as the time extends.

The amount of carbohydrate in hundred grams of egg shell and nano egg shell powder were 1.26g and 1.12g respectively . The fat content was found to be 0.22g per 100g in egg shell powder and 0.17g per 100g in nano egg shell powder. The protein content in egg shell and nano egg shell powder were 6.23g per 100g and 7.0g per 100g respectively.

Micronutrient content of egg shell powder and nanoparticles

Table II shows the micronutrient composition of egg shell powder and its nanoparticles.

The egg shell powder contains 385mg/g of calcium and the nano egg shell powder contains 420mg/g. The Slovakian egg shell powder and chicken egg shell powder appear to have a comparable calcium composition[8]. The Slovakian egg shell powder was

TABLE I
Macronutrient Composition

Nutrient	ESP (g/100g)	NESP (g/100g)
Moisture	0.349	0.26
Carbohydrate	1.26	1.12
Fat	0.22	0.17
Protein	6.23	7.0

ESP - Egg shell powder; NESP - Nano egg shell powder

TABLE II
Micronutrient Composition

Nutrient	ESP (mg/g)	NESP (mg/g)
Calcium	385	420
Magnesium	3.6	3.2
Iron	15.3	17.1
Zinc	4.5	4.7
Copper	7.7	9.0
Selenium	19.4	13.4

ESP–Egg shell powder NESP–Nano egg shell powder

found to have 386-415 mg of calcium in one gram.

The amount of magnesium in one gram of egg shell and nano egg shell powder are 3.6mg and 3.2mg respectively. The Slovakian egg shell powder contains 3.5-5.5mg/g of magnesium[8]. From this data it is evident that the magnesium content is higher in Slovakian egg shell powder than that of chicken egg shell powder analysed in the present study.

The iron content was found to be 15.3mg/g in eggshell powder and 17.1mg/g in nano eggshell powder. Schaafsma *et al* [8] experimentally proved that the iron content in Slovakian eggshell was 0.020-0.025 mg/g which is compared with the present results of chicken eggshell powder was found to be very less.

The zinc content in eggshell and nano eggshell powder were 4.5mg/g and 4.7mg/g respectively. Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 2g. Skeletal muscle accounts for approximately 60 per cent of the total body content and bone mass, with a zinc concentration of 100 - 200µg/g, for approximately 30 per cent. The proportion of copper in egg shell powder was 7.7mg/g and nano egg shell powder was 9mg/g. The selenium level in egg shell and nano egg shell powder were 19.4mg/g and 13.4mg/g respectively. Egg shell were found to be rich source of antioxidant minerals.

Conclusion

From the foregoing results, it is evident that the development of egg shell powder and eggshell nanoparticles were economical by normal blending and chemical precipitation methods respectively. Based on the characterization of the egg shell nanoparticles, it was interesting to note that the egg shell nanoparticles were in the size of 79.1nm, as evident from the particle size analyzer. Scanning Electron Microscope (SEM) images showed results in terms of spherical shape as morphology. The negative zeta potential may be attributed to the emulsion of samples with water. The Fourier Transform Infra Red Spectroscopy (FTIR) analysis showed the maximum peak intensity at 1106cm^{-1} proving the presence of carbonate and two observable peaks at 1197cm^{-1} and 912cm^{-1} for calcium carbonate. The nano egg shell powder contains more calcium than the egg shell powder. The present study proved the feasibility of preparing egg shell nanoparticles. In depth *in vitro* and *in vivo* studies are recommended for establishing its role as dietary supplement or nutraceuticals.

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