Development and Nutritional Evaluation of Eggshell Powder and its Nanoparticles

R.Hamsa Devi

(13PFN006)

Thesis Submitted to
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641043.

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Food Science and Nutrition

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Signature of the Head of the Department

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I 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 (Yasothai and Kavithaa, 2014).

Egg is a biological structure intended by nature for reproduction. It protects and provides a complete diet for the developing embryo, and serves as the principal source of food for the first few days of the chick's life. The egg is also one of the most nutritious and versatile of human foods. When the egg is freshly laid, the shell is completely filled. The air cell is formed by contraction of the contents during cooling and by the loss of moisture. A high-quality egg has only a small air cell.

The yolk is well-centered in the albumin and is surrounded by the vitelline membrane, which is colorless. The germinal disc, where fertilization takes place, is attached to the yolk. On opposite sides of the yolk are two, twisted, whitish cord-like objects known as chalazae. Their function is to support the yolk in the center of the albumin. Chalazae may vary in size and density, but do not affect either cooking performance or nutritional value.

A large portion of the albumin is thick. Surrounding the albumin are two shell membranes and the shell itself. The shell contains several thousand pores that permit the egg to "breathe." The eggshell is an important structure for two reasons. Firstly, it forms an embryonic chamber for the developing chick, providing mechanical protection and a controlled gas exchange medium. Secondly, it is a container for the market egg, providing protection of the contents and a unique package for a valuable food (Hunton, 2005).

The eggshell is comprised of five layers: an inner eggshell membrane, an outer eggshell membrane, the mammillary layer, spongy layer and cuticle. The eggshell membrane is also called “organic matrix”. This matrix is comprised of a combination of proteins and mucopolysaccharides. Most of the protein is made of keratin with a high concentration of sulfur (70-75 per cent) while about 10 per cent of protein is collagen. From the chemical point of view, the eggshell consists of water (2 per cent) and dry matter (98 per cent). The dry matter consists of five per cent crude protein and 93 per cent ash (Siske *et al*., 2007).

The superficial structure of the shell has been known for over 100 years. The shell consists of 97 per cent calcium carbonate, and this is provided to the hen in the diet. However, the chemical must be broken down in the digestive system and then re-synthesized in the shell gland to form the shell. This results in a turnover of blood calcium of approximately 100 times each 24 hours.

The eggshell is crucial because it protects the embryo from mechanical damage, and regulates gas exchange between the developing embryo and the external environment. It also prevents contamination by bacteria and other pathogens, of a very vulnerable organism. Finally, the eggshell provides a source of nutrients, primarily calcium, to the developing embryo (Hunton, 2005).

An eggshell is the outer covering of a hard shelled egg and of some forms of eggs with soft outer coats. Eggshell contains calcium carbonate and dissolve in various acids, including the vinegar used in cooking. While dissolving the calcium carbonate in an eggshell reacts with the acid to form carbondioxide (Van.physics.illinois.edu., 2007).

According to Hunton (2005), the chicken eggshell is 95 to 97 per cent calcium carbonate crystals, which are stabilized by a protein matrix. Without the protein, the crystal structure would be too brittle to keep its form and the organic matrix is thought to have a role in deposition of calcium during the mineralization process. Eggshell formation requires gram amounts of calcium being deposited within hours, which must be supplied via the hen’s diet.

Eggshells remain largely unutilised and untransformed as they are discarded wrongly. These shells are made up of calcium carbonate which is used in bone and dental therapy. The development of biomaterials for bone tissue replacements has increased and attracted a lot of interest due to the rise in the number of patients who requires bone replacements, especially in those suffering from bone cancer, trauma, and ageing. The biomaterials must be biocompatible with sufficient mechanical strength to support the weight of human body before being used as bone implants (Li and Tjong, 2011).

Egg shell waste is available in huge quantities from the food processing, egg breaking, and hatching industries. About 250,000 tons of egg shell waste is produced annually worldwide by the food processing industry only. Uronic acid, sialic acid, alanine, and glycine were high in the organic matter of egg shell waste compared to shell membranes (Nakano *et al*., 2008).

Chicken eggshell is a waste material from domestic sources such as hatcheries, poultry farms, egg product factories, homes and restaurants. Many researchers have been looking for ways to utilize the eggshell waste, for example, by using eggshell powder as a stabilizing material for improving soil properties, as a source of calcium for piglets and as a source of calcium in human nutrition. The nutritive value of eggshells showed a calcium level comparable to that of limestone, with the benefit of a small amount of protein (Gongruttananun, 2011).

Nanotechnology focuses on the characterization, fabrication, and manipulation of biological and non-biological structures smaller than 100 nm. Structures on this scale have been shown to have unique and novel functional properties. According to the National Nanotechnology Initiative (2006), "Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale."

Applications of nanotechnology within the food industry are limited. However, achievements and discoveries in nanotechnology are beginning to impact the food industry and associated industries; this affects important aspects from food safety to the molecular synthesis of new food products and ingredients (Chen *et al*., 2006).

The fact that systems with structural features on the nanoscale have physical, chemical, and biological properties substantially different from their macroscopic counterparts is changing the understanding of biological and physical phenomena in food systems. Since foods are complex biological systems that are governed by many of the same basic mechanisms and principles that biologists and biochemists study, one would expect that the discoveries made in nanotechnology may eventually also impact the food industry. However, foods undergo a variety of post harvest and processing-induced modifications that affect the biological and biochemical functionality of the system. Nanotechnology allows scientists to measure, control, and manipulate matter at the nanoscale level to change those properties and functions in a beneficial way.

Nanotechnology as a tool for achieving further advancements in the food industry are as follows: Increased security of manufacturing, processing, and shipping of food products through sensors for pathogen and contaminant detection; Devices to maintain historical environmental records of a particular product and tracking of individual shipments; Systems that provide integration of sensing, localization, reporting, and remote control of food products (smart/intelligent systems) and that can increase efficacy and security of food processing and transportation; Encapsulation and delivery systems that carry, protect, and deliver functional food ingredients to their specific site of action. Most nano technological research focuses on the development of applications in biosciences and engineering (Weiss, 2006).

Strategies to apply nanoscience to the food industry are quite different from these more traditional applications of nanotechnology. Food processing is a multi technological manufacturing industry involving a wide variety of raw materials, high biosafety requirements, and well-regulated technological processes. Four major areas in food production may benefit from nanotechnology: development of new functional materials, microscale and nanoscale processing, product development, and methods and instrumentation design for improved food safety and bio-security. The influence of the material properties of foods at the nanoscale level on their bioavailability and nutritional value has been highlighted (Aguilera, 2005).

Eggshell which is discarded has multiple benefits in terms of nutrition and availability. The eggshell is rich in calcium hence it can be used in most of the medical conditions such as osteoporosis. The eggshell can be a novel sorce 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 nanoparticle preparation and application on various health issues are not available in India. Hence the need for the present study.

The specific objectives of the study are: To

- Develop eggshell powder and its nanoparticles.
- Characterize the nanoparticles of eggshell powder
- Evaluate the nutritive value of eggshell powder and its nanoparticles.
- Compare the nutritive value of eggshell powder and its nanoparticles.

II REVIEW OF LITERATURE

The review of literature pertaining to the study entitled **“Development and Nutritional Evaluation of Eggshell Powder and its Nanoparticles”** is reviewed under the following headings:

A. Eggshell and its Composition

B. Nutritional Significance of Eggshell

C. Eggshell as Biodegradable Source

D. Health Benefits of Eggshell

E. Applications of Nanotechnology in Food

A. EGGSHELL AND ITS COMPOSITION

Eggshells are waste materials from hatcheries, homes and fast food industries and can be readily collected in plenty. Eggshell waste disposal contributes to environmental pollution. Challenges associated with disposal of eggshells include cost, availability of disposal sites, odour, flies and abrasiveness. However, they can be processed into saleable products like fertilizer, used in artwork, human and animal nutrition and building materials and to produce collagen from the membranes (Amu *et al*., 2005; Phil and Zhihong, 2009).

In Tamil Nadu, M/s. SKM Egg Products Export India Limited, Erode is manufacturing egg powder with the plant capacity of breaking 1.2 million eggs per day and has an output capacity of producing 5.5 tonnes eggshell meal per day, which is sold at relatively cheaper cost than shell grit and limestone (Yasothai and Kavithaa, 2014).

Eggshell typically consists of ceramic materials constituted by a three-layered structure, namely the cuticle on the outer surface, a spongy (calcareous) layer and an inner lamellar (or mammillary) layer. The spongy and mammillary layers form a matrix composed of protein fibers bonded to calcite (calcium carbonate) crystal. The two layers are also constructed in such a manner that there are numerous circular openings (pores). This structure permits gaseous exchange throughout the shell. The outer surface of the eggshell is covered with a mucin protein that acts as a soluble plug for the pores in the shell. The cuticle is also permeable to gas transmission.

The egg possesses two major natural defense systems. The first one is the eggshell, together with the cuticle and membranes, which constitute a physical barrier against bacterial penetration. The integrity of this structure is crucial for the protection of the contents of the egg from microbial environment and in the control of water and gases through the pores of the shell during the embryonic development. Defects in the mineralized shell are directly related to increasing risk of bacterial penetration in the egg. The second natural defense of the egg is a chemical barrier composed of proteins that exhibit anti-microbial activity found in the albumin and at a lesser extent in the other compartment of the egg yolk and eggshell (Gautron and Nys, 2006).

The chemical composition (by weight) of by-product eggshell has been reported as follows: calcium carbonate (94 per cent), magnesium carbonate (1 per cent), calcium phosphate (1 per cent) and organic matter (4 per cent) (Stadelman, 2000). Notably, the by-product eggshell generated from food processing and manufacturing plants is inevitably composed of calcium carbonate (eggshell) and eggshell membrane. The eggshell membrane resides between the egg white (albumin) and the inner surface of the eggshell. There are two shell membranes around the egg, a thick outer membrane attached to the shell and a thin inner membrane (Nakano *et al*., 2008). Figures 1 and 2 show the structure of egg and eggshell respectively.

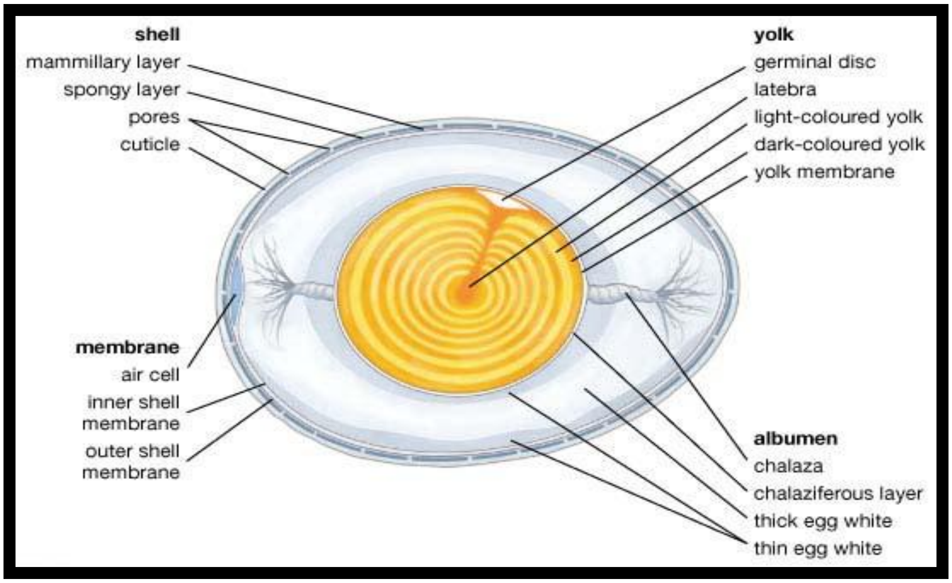


FIGURE 1

STRUCTURE OF EGG

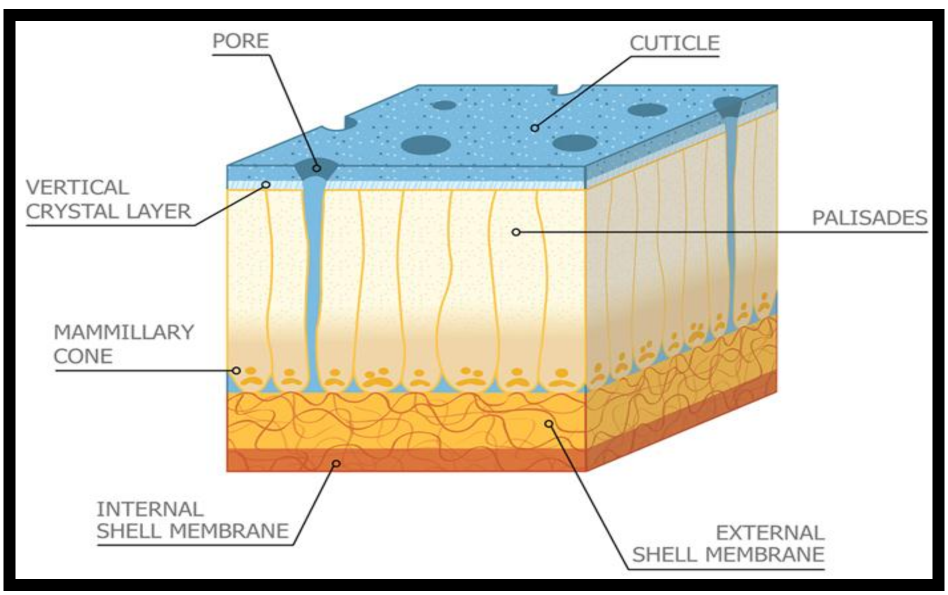


FIGURE 2

STRUCTURE OF EGGSHELL

The total thickness of these two membranes has been found at approximately 100 lm. Each of these membranes is composed of protein fibers that are arranged so as to form a semi-permeable membrane. Therefore, the eggshell membrane possesses an intricate lattice network of stable and water-insoluble fibers and has high surface area resulting in various applications such as adsorbent (Allen *et al*., 2000; Gota and Suyama, 2000; Koumanova *et al*., 2002) and immobilization support (Xiao and Choi, 2002; Yang *et al*., 2003; Choi and Yiu, 2004).

Shell membranes consist of collagen as a component. The collagen is extracted and has diverse uses in medicine, biochemical, pharmaceutical, food and cosmetic industries. These uses minimize their effect on environmental pollution. The eggshell and shell membranes make up 10.2 per cent of the whole egg. The eggshell comprises of calcified shell and shell membranes including inner and outer membranes. MacNeil (1997) developed a patent for separating egg shell membranes from the eggshell.

The organic matter of eggshell and shell membranes contains proteins as major constituents with small amounts of carbohydrates and lipids (Burley and Vadehra, 1989). The composition of the egg shell is approximately 98.2, 0.9, 0.9 per cent calcium carbonate, magnesium and phosphorous (phosphate) respectively (Romanoff *et al*., 1949). Shell membranes comprises of 69.2 per cent protein, 2.7 per cent fat, 1.5 per cent moisture and 27.2 per cent ash (MacNeil, 1997). Shell membranes protein comprises of approximately 10 per cent collagen (Froning, 1998). Eggshell and shell membranes are non-edible by-products with little saleable value but they may contain biologically active compounds (Nakano *et al*., 2008).

B. NUTRITIONAL SIGNIFICANCE OF EGGSHELL

Most good quality eggshells from commercial layers contain approximately 2.2 grams of calcium in the form of calcium carbonate. About 95 per cent of the dry eggshell is calcium carbonate weighing 5.5 grams (10-11 per cent of egg weight). The average eggshell contains about 94-97 per cent calcium carbonate, 0.3 per cent phosphorus and 0.3 per cent magnesium and traces of sodium, potassium, zinc, manganese, iron and copper.

An eggshell that is smooth is desirable as rough shelled eggs fracture more easily. Large sized eggs will usually break more easily than small ones. The main reason for this is that the hen is genetically capable of placing only a finite amount of calcium in the shell.

Several calcium sources are available for food fortification. Calcium carbonate is the most widely used calcium salt because 40 per cent of the compound is well absorbable calcium. This calcium salt can be formulated from calcium hydroxide or chalk in the laboratory but can also be derived from fossilized or fresh shells (e.g., chicken eggshell and oyster shell). Natural calcium sources are of interest because they contain not only calcium but also other elements (e.g. strontium and fluorine), which may have a positive effect on bone metabolism. The safety of natural calcium supplements, however is doubtful because they may contain relevant amounts of potential toxic element such as lead, aluminium, cadmium, and mercury (Nakano *et al*., 2008).

The mineral calcium plays a major role in bone strength and is of prime nutritional importance in osteoporosis, being essential for bone health throughout life. The primary role of calcium in the body is structural, providing the rigidity necessary for the skeleton and teeth to function mechanically. Bone contains about 99 per cent of the body’s calcium.

Calcium in body fluids also exerts critical metabolic functions, binding to proteins, and operating as a signal transmitter and protein activator within cells. Muscle contraction and nerve transmission are two of the many body functions that rely on calcium for activation. Additionally, calcium is also involved in blood clotting (Heaney, 2009).

Calcium is required for normal growth and development of the skeleton. Adequate calcium intake is critical for achieving optimal peak bone mass and modifies the rate of bone loss associated with aging (National Osteoporosis Foundation, 2008). Over the past decade, convincing evidence has emerged with respect to the effects of dietary calcium on bone health in all age groups. Unfortunately, there is a significant proportion of some population groups failing to achieve the recommended calcium intakes in a number of Western countries (Cashman, 2002).

Protein is also a key constituent of bone tissue and therefore an adequate dietary supply is essential. The majority of the observational studies support a positive association between protein intake and bone health. There are several epidemiological studies, both cross-sectional and longitudinal that have reported an association between dietary protein and bone. These studies reveal that individuals who consume the most dietary protein have the highest bone mineral density. In addition, prospective studies have observed that individuals with the highest protein intake have the slowest rate of bone loss (Hannan *et al*.,2000; Kerstetter *et al*., 2000; Promislow *et al*.,2002; Rapuri *et al*.,2003).

Chicken eggshells, which first serve to protect and provide nutrients to the enclosed embryo, have been used by humans for a long period as a food additive, but on a very modest scale. The chicken eggshell comprises calcified shell and shell membranes including inner and outer membranes. These membranes retain albumin and prevent penetration of bacteria. Shell membranes are also essential for the formation of eggshell.

The organic matter of eggshell and shell membranes contain proteins as major constituents with small amounts of carbohydrates and lipids. Uronic acid is a constituent sugar of glycosaminoglycan. The organic matter of eggshell contains two glycosaminoglycans including hyaluronic acid and chondroitin sulfate-dermatan sulfate copolymer. Sialic acid is another carbohydrate found in eggshell membranes. However, little is known about the difference in concentrations of sialic acid between the inner and outer eggshell membranes. There is also limited information available concerning variations in nitrogen concentrations and amino acid composition among the organic matter of eggshell and shell membranes.

Eggshell and shell membranes are non-edible by-products with little saleable value. However, they may contain biologically active compounds. Better understanding of chemical composition of these by-products is of basic importance. Such knowledge may also be important for investigating the biological role of eggshell and shell membranes (Nakano *et al*., 2008).

C. EGGSHELL AS BIODEGRADABLE SOURCE

Agricultural waste is any waste being generated from different farming processes in accumulative concentration. Adequate utilization of agricultural waste reduces environmental problems caused by irresponsible disposal of the waste. The management of agricultural wastes is indispensable and a crucial strategy in global waste management. Waste of any kind in the environment when its concentration is in excess can become a critical factor for humans, animals, and vegetation (Seadi and Holm-Nielsen, 2004).

The nature, quantity, and type of agricultural waste generated vary from country to country. The search for an effective way to properly manage agricultural waste will help protect the environment and the health quality. For sustainable development, wastes should be recycled, reused, and channelled towards the production of value added products. This is to protect the environment on one side and on the other side to obtain value added products while establishing a zero waste standard. The utilization of the waste is a priority today in order to achieve sustainable development (Yates *et al*., 2011).

One way that adds great value to agricultural waste is its utilization as a biomaterial used in medical surgery and therapeutics. The production of biocompatible material or biomaterial from agrowaste has added a different dimension to the utilization of agricultural waste for value added product. This is possible because some of this waste contains active compounds that have value in medical applications.

This is a novel practice that is expected to have value in medical sciences. Most researches on agricultural waste focused mainly on its energy potentials or its use as effective chemical feedstock and as renewable raw materials because of its abundance, its cheap availability, and its renewability (Peng *et al*., 2000; Zakaria *et al*., 2010; Ling and Teo, 2011; Surip *et al*., 2012). This conversion into valuable products or energy sources is carried out by microorganisms or their components (Gioia *et al*., 2011).

Many agricultural wastes were reported to be effective feedstock in making useful products (Xu *et al*., 2011; Rashidi *et al*., 2012; Boonpoke *et al*., 2013). The waste is readily available and cheap. Agricultural waste has been proven to serve as a good replacement option which can be used as biomaterials in therapies that replaces bone for the growth of osteoblasts (Martin-Luengo *et al*., 2011).

Discarded eggshells are often used as a plant fertilizer. This is because eggshells contain calcium. Making eggshell fertilizer is inexpensive and environmental-friendly, since the process reuses material to promote plant growth. Ground eggshells are effective liming sources (John and Paul, 2006). Calcium raises, or neutralizes, the pH level of overly acidic soil. Most plants prefer slightly acidic soil with a pH between 5.8 and 7.0. A study revealed that red clover plants fertilized with eggshells grew at an average of more than 10 mm larger than plants without eggshells (Planting Science.org, 2011).

Sanitized eggshells can be used to increase mineral content of compost and spread around plants to deter slugs and snails. They are also used by artists to make mosaics and to make textured paint for three dimentional effects in artwork (Phil and Zhihong, 2009).

Most of the waste was commonly disposed of for landfill without any pretreatment. It is obvious that these approaches are not desirable practices in view of the odor from biodegradation. Occasionally, few of the by products were reused as a fertilizer or soil conditioner because of their high nutrition contents such as calcium, magnesium and phosphorus. Based on the bioresource recovery and reuse, the utilization of this food processing by-product has slightly increased in recent years (Tsai *et al*., 2006).

D. HEALTH BENEFITS OF EGGSHELL

Eggshells contain calcium and trace amounts of other micro elements, i.e. magnesium, boron, copper, iron, manganese, molybdenum, sulphur, silicon and zinc. Eggshell calcium is probably the best natural source of calcium and it is about 90 per cent absorbable (Bee, 2011). It is a much better source of calcium than limestone or coral sources.

The composition of an eggshell is very similar to that of our bones and teeth. It is recommended that people with osteoporosis take 400-500 mg calcium per day to supplement dietary sources. The powder should be taken together with some added magnesium, zinc, vitamin D3, K1, K2, strontium and boron for efficient utilization.

Calcium is a very important building block of bone and often seen as key element in bone mineralisation and anti-demineralisation strategies. Dairy products which are known as the major source of calcium are only about 10,000 years ago, introduced in the human diet. Before that time, the stone-age adult consumed at high amount of calcium form other sources, probably including egg shell. To support increased requirements or to overcome low intakes, several calcium sources are available for food enrichment. In post menopausal women and elderly, at number of calcium sources, such as purified calcium carbonate, calcium citrate, mineral complex, oyster shell electrolysate, calcium lactate gluconate, and milk-calcium have been studied for their effects on bone mineral density.

In general, the effects were comparable, causing at small, sometimes transient increase in bone mineral density or an inhibition of bone loss. Chicken egg shell powder, not commonly used as calcium source for humans, might have at higher impact on bone mineral density as has been suggested by a Slovakian study with osteoporotic patients.

The major component of egg shell powder is calcium carbonate (about 98 per cent of weight) whereas other minerals which are of interest in bone metabolism such as strontium and fluoride, are present in small amounts. Furthermore, it has been suggested by Slovakian researchers that eggshell powder contains bioactive substances. Combinations of calcium with vitamin D and magnesium enhance calcium effects on bone mineral density in postmenopausal women in case their intakes are limited.

Schaafsma *et al* (2002) reported a highly positive effect of eggshell calcium supplementation (with added magnesium and vitamin D) on bone mineral density. During this supplementation, the intervention group consumed twice daily a dairy-based which contains nearly 1800mg of calcium. This study was done in the Netherlands. The eggshell supplemented group had measurable increases in bone density in their hip bones, after one year. The findings indicate that healthy late post-menopausal women with an adequate calcium intake at baseline may increase bone mineral density of the hip within 12 months following supplementation with the chicken eggshell powder-enriched supplement.

Manganese and zinc are involved in egg shell formation and enzymatic activity at uterus level where calcification begins. Zinc as organic form is associated with the increased activity of carbonic anhydrase, improving the quality of egg shell and the presence of manganese has an activator influence on the calcification and resistance of the egg shell (Ceylan and Scheideler, 1999).

Extrusion technology has been used to utilize eggshells in laying rations (Froning and Bergquist, 1990). In laying hens feeding, organic sources of micro elements are frequently used. They seem to have a higher biological activity and bioavailability in the hen compared to inorganic sources. Lara *et al* (2007) reported that offering an organic mineral supplement (Eggshell - 49) to hybrid laying hens between 49 to 69 weeks of age had favorable effects on the main production indices and quality of egg shells. This was attributed to the role and contribution of the micro elements (manganese, zinc) and macroelements (calcium, phosphorus) present in the Eggshell - 49 supplement in the formation and improvement of the egg shell quality.

In Japan, researchers studied a combination of vitamin D3 and eggshell powder in animals with osteoporosis. The eggshell powder with vitamin D3 was able to improve bone mineral density without significantly increasing blood calcium levels. Any kind of eggshells (chicken, goose and duck) can be used, but it is best to use shells from birds that get balanced minerals in the diet (King'ori, 2011).

Rivera *et al* (1999) reported a novel procedure to porous hydroxyapatite from eggshells. Moreover, there have been studies aiming at the calcium supplement and other nutrition sources from the albumin, membrane and matrix of the eggshell, which was processed by crushing and milling (Tsai *et al*., 2006).

Lichtenstein (1948) mentioned a large store of calcium that was thrown away instead of being used for human nutrition. The chicken eggshell powder was shown to have antirachitic effects in rats. In vitro, eggshell powder stimulated the growth of chicken embryo cartilage cells. The demembranized chicken eggshell powder was studied in an elderly population with osteoporosis. Use of the eggshell powder resulted in decreased pain and increased bone mineral density.

In 1995, comparable results were obtained from a Dutch pilot study with osteoporotic subjects. In piglets, the apparent absorbability of calcium from eggshell powder was found to be at least as good as that from purified calcium carbonate (Schaafsma and Beelen, 1999). Because it has been suggested that hormone-like activity is present in eggshell powder, this might play a role in the effects on bone mineral density and pain as mentioned before. Transforming growth factor-β1 (TGF-β1) is thought to play a role in regulating bone mineral density. Calcitonin inhibits osteoclasts and reduces pain in subjects with a high bone turnover.

Based on the amino acid sequence, it has been suggested that chicken calcitonin might be one of the most potent calcitonins, and it is also effective in mammalian systems. Of the steroid hormones, both progesterone and estrogen reduce bone resorption in postmenopausal women, and progesterone may also stimulate bone formation. Calcitriol, which is the most active vitamin D3 hormone, reduces serum parathyroid hormone and, as a consequence, reduces bone turnover and loss (Schaafsma *et al*., 2000).

E. APPLICATIONS OF NANOTECHNOLOGY IN FOOD

The term 'nanofood' describes food that has been cultivated, produced, processed or packaged using nanotechnology techniques or tools, or to which manufactured nanomaterials have been added. Nanofood has, in fact, been part of food processing for centuries, since many food structures naturally exist at the nanoscale. The purpose of nanofood is to improve food safety, enhance nutrition and flavor, and cut costs. Although nanofood is still in its infancy, nanoparticles are now finding application as a carrier of antimicrobial polypeptides required against microbial deterioration of food quality in the food industry (Joseph and Morrison, 2006).

The current nanotechnology applications in food science provide the detection of food pathogens, through nanosensors that are quick, sensitive and less labor-intensive procedures. However, it is well known that the nanoparticles equipped with new chemical and physical properties that vary from normal macro particles of the same composition may interact with the living systems thereby causing unexpected toxicity. So far, warnings about nanofoods–products made via the manipulation of molecules–have not reached a tipping point in terms of public attention. Untested nanotechnology is being used in more than 100 food products, food packaging and contact materials currently on the shelf, without warning or new FDA testing (Das *et al*., 2009).

A starch-like nanoparticle can help stop lipids from oxidizing and therefore improve the stability of oil-in-water emulsions. The health benefits of curcumin, the natural pigment that gives the spice turmeric its yellow colour, could be enhanced by encapsulation in nanoemulsions.

Probiotics are products aimed at delivering living, potentially beneficial, bacterial cells to the gut ecosystem of humans and other animals. A variety of calcium and probiotic nanofood capsules, liquids, and powders are available. Powders can be stirred into food but should not be added to food warmer than room temperature, because heat will kill the bacteria. Another option is to add to the diet of people. These foods include yogurt, kefir (a cultured-milk beverage), tempeh (made from soybeans), and kimchi (a Korean fermented cabbage dish) (Renton, 2006).

A list of food products currently containing nanoproducts include: Canola Active Oil (Shemen, Haifa, Israel), Nanotea (Shenzhen Become Industry Trading Co. Guangdong, China), Fortified Fruit Juice (High Vive.com, USA), Nanoceuticals Slim Shake (assorted flavors, RBC Lifesciences, Irving, USA), NanoSlim beverage (NanoSlim), Oat Nutritional Drink (assorted flavors, Toddler Health, Los Angeles, USA), and 'Daily Vitamin Boost' fortified fruit juice (Jamba Juice Hawaii, USA) and nanocapsules containing tuna fish oil (a source of omega 3 fatty acids) in "Tip-Top" Up bread (Enfield, Australia).

A number of recent reports and reviews have identified the current and short-term projected applications of nanotechnologies for the food sector

(Kuzma and VerHage, 2006; Bouwmeester *et al*., 2007; Groves, 2008; Morris, 2008; Grobe *et al*., 2010). The main areas of application include food packaging and food products that contain nano sized or nano encapsulated ingredients and additives. The potential for food nanotechnology applications seems unlimited. All facets of the food industry from ingredients to packaging to food analysis methods are already looking into nanotech applications. These are resulting in numerous promising applications for improved food production, processing, packaging, and storage.

Applications of nanotechnology in organic food production require precaution, as little is known about their impact on environment and human health (Kang *et al*., 2007; Chau *et al*., 2007). Some recent food applications of nanotechnology, safety and risk problems of nano materials, routes for nanoparticles entering the body, existing regulations of nanotechnology in several countries, and a certification system of nano products were reported (Sozer and Kokini, 2009). Increasing uses of tools and techniques developed by nanotechnology to detect carcinogenic pathogens and biosensors for improved and contamination free food have been reported (Shrivastava and Dash, 2009).

Over the past few decades, the evolution of a number of new science disciplines and technologies has revolutionised the food sector. Most notable among these are biotechnology, cognitive sciences, information technology (IT) and, more recently, nanotechnology, which is a broad interdisciplinary area of research, development and industrial activity that involves the manufacture, processing and application of materials that have one or more dimensions of the order of 100 nanometers (nm) or less (British Standard Institute, 2005).

Nanotechnology is an enabling technology that has opened up new avenues of research and development in a number of fields, including medicine, cosmetics, agriculture and food, and is being used as a means to understand how physicochemical characteristics of nano-sized substances can change the structure, texture and quality of foodstuffs.

The applications of nanotechnology in the food sector are only new emergent, but they are predicted to grow rapidly in the coming years. Many of the world's largest food companies are reported to have been actively exploring the potential of nanotechnology for use in food or food packaging (Cientifica, 2006; The Sunday Telegraph, 2006).

The main concerns stem from the lack of knowledge with regard to the interactions of nano-sized materials at the molecular or physiological levels and their potential effects and impacts on consumer's health and the environment.

For centuries, foods have been processed to enhance storage (preservation), texture, flavour, taste and nutritional value, and to ensure microbiological safety. Most modern-day foods undergo a variety of industrial and domestic processes before being eaten, e.g. heat-treatment, fermentation, acid hydrolysis, kilning, curing, smoking and drying.

The food industry is also looking out for new technologies to improve the nutritional value, shelf-life and traceability of their food products. They are also aiming to develop improved tastes, reduce the amount of salt, sugar, fat and preservatives, address food-related illnesses (e.g. obesity and diabetes), develop targeted nutrition for different lifestyles and aging population, and maintain sustainability of food production, processing and food safety (Cientifica, 2006).

A number of new processes and materials derived from nanotechnology can provide answers to many of these needs, as they offer the ability to control and manipulate properties of substances close to molecular level. For example, in terms of increasing the absorption of nano-sized nutrients and supplements and, therefore, enhancing the nutritional value of food, development of new tastes/sensations and creamier textures through nano structuring of food ingredients with less or no additional fat.

Although nanotechnology applications for the food sector are relatively recent, there have been rapid developments in this area in recent years. The main developments so far been aimed at altering the texture of food components, encapsulating food components or additives, developing new tastes and sensations, controlling the release of flavours, and/or increasing the bioavailability of nutritional components.

A major focus of current nanotechnology applications in food is the development of nanostructured (or nanotextured) food ingredients and delivery systems for nutrients and supplements. Another major area of current nanotechnology applications is nanoencapsulation of food ingredients and additives (Chaudhry *et al*., 2008).

Food nanotechnology advances offers important challenges for both government and industry. The food processing industry must ensure consumer confidence and acceptance of nanofoods. Regulatory bodies, such as FDA, should author guidance with respect to the criteria to be followed in evaluating the safety of food, food packaging, and supplement uses of nanomaterials with novel properties (Sekhon, 2010).

III METHODOLOGY

The methodology adopted for the present study entitled **"Development and Nutritional Evaluation of Eggshell Powder and its Nanoparticles"** is dealt under the following phases:

PHASE I: DEVELOPMENT OF EGGSHELL POWDER AND ITS NANOPARTICLES

A. Selection of Eggshell
B. Development of Eggshell Powder
C. Development of Eggshell Nanoparticles

PHASE II – CHARACTERIZATION OF EGGSHELL NANOPARTICLES

D. UV Visible Spectroscopy Analysis
E. Particle Size Analysis
F. Scanning Electron Microscope (SEM) Analysis
G. Zeta Potential Analysis
H. Fourier Transform InfraRed Spectroscopy (FTIR) Analysis

PHASE III – EVALUATION OF NUTRIENT CONTENT

I. Nutrient Content of Eggshell Powder and its Nanoparticles

PHASE I: DEVELOPMENT OF EGGSHELL POWDER AND ITS NANOPARTICLES

A. Selection of Eggshell

The eggshell is an important structure for two reasons. Firstly, it forms an embryonic chamber for the developing chick, providing mechanical protection and a controlled gas exchange medium. Secondly, it is a container for the market egg, providing protection of the contents and a unique package for a valuable food.

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.

Other possibilities for utilising egg shell include: production of biodegradable plastics from egg shell membrane proteins; altering of food-borne bacterial pathogen heat resistance with an egg shell membrane bacteriolytic enzyme; as human dietary calcium supplement especially for post menopausal women. Eggshells also contain useful amounts of microelements such as strontium (Sr), fluorine (F) and selenium (Se); it's membrane can be used as an adsorbent for the removal of reactive dyes from coloured waste effluents as well as to eliminate heavy metal ions from a dilute waste solution (Adeyeye, 2009).

Very early in the study of the shell, chemical analyses showed that it is composed of about 97 per cent calcium carbonate, (Wiley and Sons, 1989) and most of the research on shell quality concentrated on this fact. This is because calcium has to be provided as part of the hens diet, while the carbonate portion is produced internally during the normal course of metabolism.

The dietary ingredient normally used to provide calcium to the laying hen is usually calcium carbonate the same as the shell. However, this feed ingredient is broken down into its components in the intestine, and the calcium is absorbed into the blood stream. It is then either stored in the bones until needed for shell formation, or transported directly to the shell gland for synthesis into eggshell calcium carbonate. Shell formation is by far the lengthy process in egg formation. Once the ovum, with the germ cell attached, is released from the ovary, it takes roughly 4-6 hours to lay down the albumin, and 18-20 hours to laydown the membranes and the shell (Etches RJ, 1987).

The eggshell required for the study was collected from the local bakery in fresh basis. The quantity of the eggshell used by the bakery per day was collected which was one kilogram in weight. 500g of eggshell was used for the development of eggshell powder and 500g of eggshell was used for the development of eggshell nanoparticles.

B. Development of Eggshell Powder

Empty eggshells were washed in warm water until all of the egg white was removed, but the membrane was not removed because it contains important nutrients for the joints which helps arthritis. Broken pieces was layed out on paper towels and allowed to air dry thoroughly.

The eggshells were broken into small pieces, and initially ground in a mortar and pestle. Further it was ground into a fine powder in a food processor/ blender. Care should be taken to grind the eggshell into a fine powder. The powdered eggshells was stored in a covered glass jar or container and kept it in a dry place.

Eggshells present healthy, balanced calcium due to trace amounts of other minerals contained in it. Eggshell calcium is probably the best natural source of calcium, and it is easier for our body to digest and absorb. Dutch researchers have reported recently a highly positive effect of eggshell calcium (with added magnesium and vitamin D) on bone mineral density in a scientific study (double blind, placebo-controlled) (King'ori, 2011). Plate 1 shows the stages wise preparation of Eggshell powder.

UNCRUSHED EGGSHELL

CRUSHING OF EGGSHELL POWDER

EGGSHELL POWDER

PLATE 1

DEVELOPMENT OF EGGSHELL POWDER

C. Development of Eggshell Nanoparticles

The eggshell nanoparticles were prepared by the method of chemical precipitation via aqueous slurries comprising of biowaste eggshells and phosphoric acid solution. In the first step, uncrushed and washed raw eggshells were collected and mechanically cleaned and powdered (Plate 2). This was followed by washing alternatively with 100 ml of distilled water and 100 ml of acetic acid for a total of three times each followed by a final water wash (Plate 3).

The treated eggshells were then placed in an oven for a three stage thermal treatment. These were kept at 150˚C for five hours (to remove the water and acid), 500˚C for three hours (to remove any remaining organic residue) and finally 1000˚C for two hours to transform the eggshells to calcium oxide (Plate 4).

The calcium oxide from the heat-treated eggshells was transformed into hydroxyapatite in a phosphate solution using the following procedure; 100g of calcium oxide from cleaned and heat treated eggshells were dissolved under stirring in 119g phosphoric acid, then 7g of urea was added along with 15g of ethylene diamine tetra acetic (EDTA) acid. This was then added to 1600ml of distilled water to form a solution. The pH of the solution was then adjusted to 10 (Plate 5) using ammonia solution at 95˚C. The reaction mixture was kept for 24 hours to allow nanocrystalline growth.

After incubation, the slurry was centrifuged (500pm for two minutes) (Plate 6) and the resulting sludge was thoroughly washed with distilled water until the pH value decreased to 7 after re-centrifuging. The resulting sludge was homogeneously suspended in distilled water and filtered using Buchner funnel (20cm) with application of mild suction (Plate 7).

After filtration, precipitate was dispersed in 100ml ethanol (and ultrasonicated for 1hour) and then dried in air for 24 hours. Plate 8 shows the ultrasonication of the precipitate. The powder was then further dried at 400˚C, 500 to 1200˚C (in 100˚C intervals) using a muffle furnace and a heating and cooling rate of 10˚C $min^{-}1$ with a two hour holding time at each target temperature. Thus, the nanoparticles of eggshell were prepared (Plate 9). 500g of uncrushed eggshell was taken and 30g of nanoparticles were obtained.

PLATE 2

EGGSHELL POWDER USED FOR NANOPARTICLE DEVELOPMENT

PLATE 3

WASHING THE POWDER WITH ACETIC ACID

PLATE 4

FORMATION OF CALCIUM OXIDE

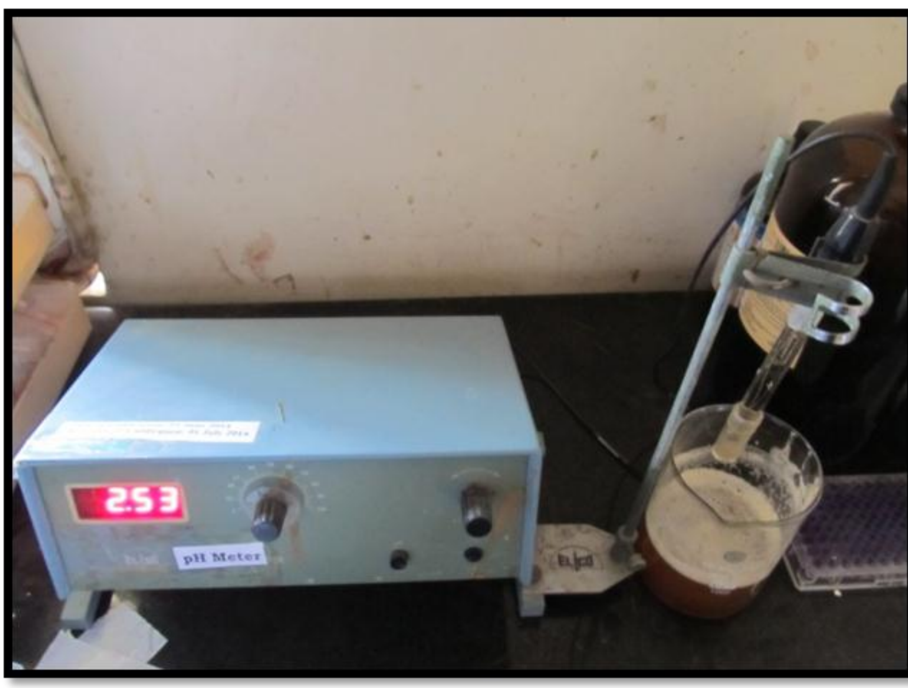


PLATE 5

ADJUSTING THE pH

PLATE 6

CENTRIFUGATION AT HIGH SPEED

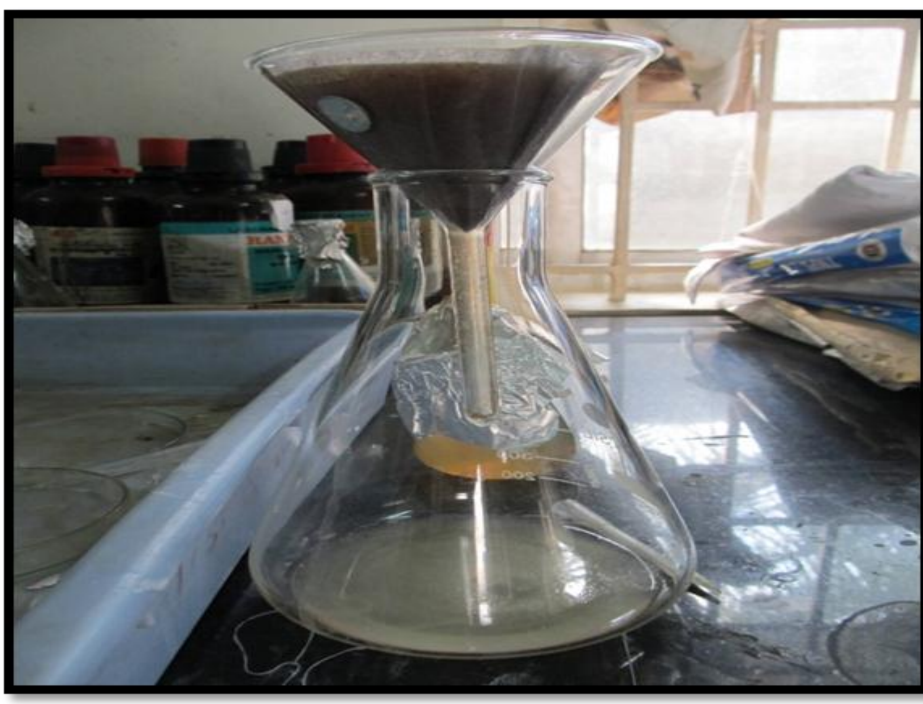


PLATE 7

FILTRATION

PLATE 8

ULTRASONICATION

PLATE 9

EGGSHELL NANOPOWDER

PHASE II – CHARACTERIZATION OF EGGSHELL NANOPARTICLES

D. UV Visible Spectroscopy Analysis

Ultraviolet–visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

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 of light it can absorb. The synthesized eggshell nanoparticle were initially characterized with UV-visible spectrophotometer (Plate 10). Absorption measurements were carried out on UV - specord 210 plus at a resolution of 1nm. UV visible analysis ranges from 200 to 600 nm.

E. 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. In order to simplify the measurement process, it is often convenient to define the particle size using the concept of equivalent spheres. In this case, the particle size is defined by the diameter of an equivalent sphere having the same property as the actual particle such as volume or mass for example. It is important to realize that different measurement techniques use different equivalent sphere models and therefore will not necessarily give exactly the same result for the particle diameter.

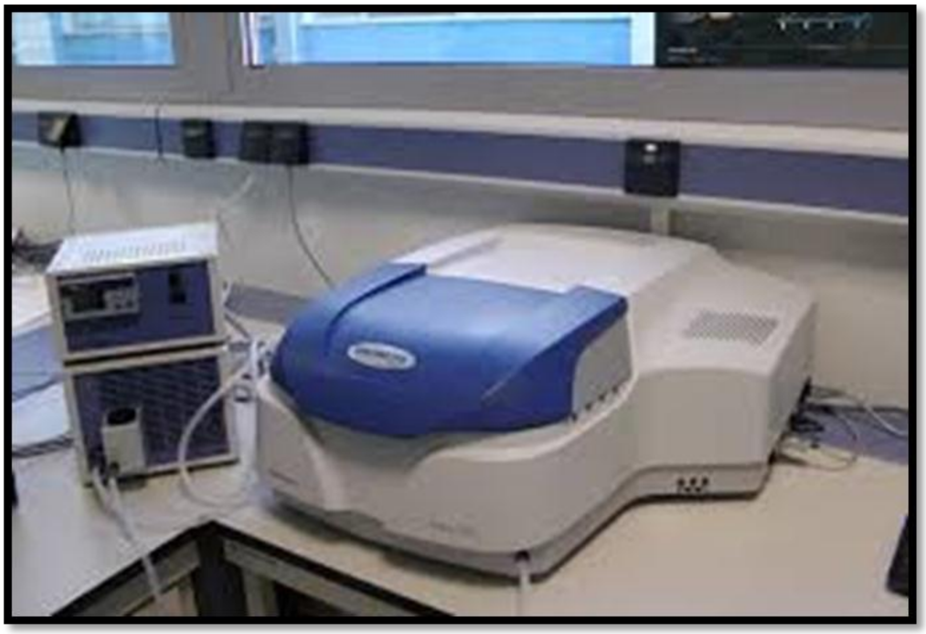


PLATE 10

UV VISIBLE SPECTROSCOPY

PLATE 11

PARTICLE SIZE AND ZETA POTENTIAL ANALYSER

The particle size analysis was performed in SZ-100 Nanoparticle Size Analyzer (Plate 11). 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.

F. Scanning Electron Microscopic (SEM) Analysis

A scanning electron microscope 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 electrons interact with the atoms that make up the sample producing signals that contain information about the sample surface topography, composition and other properties such as electrical conductivity.

The surface topography of nanoparticles was observed with a Scanning Electron Microscope (SEM). These were characterized using Jeol Model - 6390 Scanning Electron Microscopy (SEM) (Plate 12). 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.

G. 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, or speeding particle flocculation for water treatment for example. Measuring zeta potential by electrophoretic light scattering allows one to assess the effects of various strategies for manipulating zeta potential. Electrophoretic light scattering exploits the fact that a charged particle responds to an applied electric field.

From the known applied electric field and measured particle velocity, the particle mobility is readily determined. Zeta potential is then calculated from mobility by using a model, the most common of which is the Smoluchowski model. The only parameters required for determining zeta potential are liquid dielectric constant, refractive index, and viscosity. This makes the technique rapid and reliable.

The zeta potential analysis was performed in SZ-100 Nanoparticle 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.

The measurements are 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.

H. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

Fourier Transform InfraRed Spectroscopy (FTIR) spectrum of samples was recorded on ATR-FTIR brucker alpha (tramission) instrument (Plate 13) 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.

PLATE 12

SCANNING ELECTRON MICROSCOPIC (SEM) ANALYSIS

PLATE 13

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

PHASE III: EVALUATION OF NUTRIENT CONTENT

I. Nutrient Content of Eggshell Powder and its Nanoparticles

a. Moisture Estimation

Estimation of moisture is one of the most often performed determinations in food analysis. Moisture is lost when food is heated not much higher than the temperature of boiling water or by allowing to stand overnight over dehydrating agent or by heating over vacuum.

Heated a pair of weighing bottles at 100° c in oven and labeled A and B. Placed on an asbestos sheet for 2 minutes and then transferred them to a dessicator where they remained for half an hour. Recorded their weights in an analytical balance. Repeated this procedure till two successive weights obtained were constant (with maximum difference of 0.0002g).

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 dessicator for half an hour and weighed. This was also weighed till successive weighings showed no further loss.

b. Carbohydrate Estimation

Carbohydrates are hydrolyzed into sample sugar using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This compound forms with anthrone, a green colour with an absorption maximum at 630nm.

Weighed 100mg of sample into a boiling tube. Hydrolysed by keeping it in boiling water bath. Boiled for three hours with 5ml 2.5N HCL and cool to room temperature. Neutralize it with solid sodium carbonate until effervescence case. Made up the volume to 100ml and centrifuge. Collected the supernatant and 0.5ml and 1ml aliquots used for analysis. Prepared the standard by taking 0.0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard ‘0’ serves as blank. Make up the volume to 1ml in all the tube including the sample tubes by adding distilled water. Then added 4ml of anthrone reagent and heat to 8 minutes in boiling water bath. Cool rapidly and read the green to dark green colour at 630nm. Draw a standard graph by plotting concentration of the standard on the x axis verses absorbance on the y axis. From the graph calculate the amount of carbohydrate present in the sample tube.

c. Fat Estimation

The soxhlet extraction used depends on the intermittent action a glass siphon. The ether gradually condenses into the extraction tube containing the material until it rises to the top when it is discharged into the extraction flask.

The soxhlet flask was weighed to consecutive concordant weights. 2g of the moisture free sample was packed into an extraction thimble and placed in an extractor which was fixed into a soxhlet flask. Poured sufficient amount (150 ml) of petroleum ether so as to permit siphon action. The thimble and the contents were allowed to soak in ether for 24 hours. The entire set up was kept over an electric water bath and the extractor was connected to the condenser. The nozzle of the condenser was always plugged with moistened cotton. The temperature was maintained at 60°c. a steady stream of water in the condenser was maintained. The ether evaporated rose up but owing to the condenser arrangement, it fell back into the condenser extractor. When the extractor got filled with ether, it was siphoned back into the flask. This went on till the ether that got collected in the extractor was free from any yellow colour indicating the presence of fat. The soxhlet flask was then disconnected and ether was evaporated in a water bath maintained at 60°c. When the ether in the flask was evaporated, the flask was weighed again to get concordant values. From the difference in weight, the fat content was calculated.

d. Total Protein Estimation

The given sample is digested with concentrated sulphuric acid in a macrokjeldahl flask when nitrogen gets converted to ammonium sulphate. Ammonia is liberated by the action of strong alkali in a macrokjeldahl steam distillation apparatus. This nitrogenous substance is converted to ammonium borate by absorbing 2 per cent boric acid and is titrated against N/70 H_2SO_4. The volume of acid required to bring the test sample to the colour of the blank gives the acid equivalent to the ammonia.

PLATE 14

FAT ESTIMATION

PLATE 15

PROTEIN ESTIMATION

0.5g of the sample was taken into the digestion flask. To this added 15 ml of concentrated sulphuric acid and a pinch of digestion mixture as a catalyst. Kept at boiling gently over a heating mantle. After digestion, the flask was cooled and the contents were transferred to a 100 ml standard flask and made upto the mark with distilled water. The whole apparatus was washed with distilled water and allowed to back suck. 10ml of boric acid was taken in a conical flask. A drop of indicator was added to it and kept under the condenser. The tip of the condenser was well below the liquid.

5ml of the digested blank was added into the Distillation chamber through the funnel. Then added 10ml of 40per cent NaOH. Washed the funnel with 2 to 3 ml of distilled water. Closed the tap and steam was generated. Steam entered the distillation chamber and drove all the ammonia which is inturn absorbed by boric acid. Solution was pinkish white in colour, turned blue. Steam was passed for 5 min and then the conical flask was lowered and the tip of the condenser washed. The boric acid solution containing the liberated ammonia was titrated against N/70 H_2SO_4. The end point was the appearance of pale permanent pink colourBetween each estimation, the apparatus was washed. The experiment was repeated to get concordant values.

e. Calcium Estimation

Calcium is determined by precipitating it as calcium oxalate and titrating the oxalate solution in dilute sulphuric acid against standard potassium permanganate.

Ash from the ignited sample was dissolved in hydrochloric acid and made upto the 100 ml. 10 ml of the ash solution was pipetted out in a conical flask and 90 ml of distilled water was added to it. Added 2 drops of methyl red indicator. It was made strongly alkaline by adding ammonia (colour-yellow) and kept for boiling for minutes. 20 ml of saturated ammonium oxalate was added to the solution, 10 ml of each time to ensure complete precipitation directly. When it was hot, a few drops of acetic acid was added to render the medium acidic (colour pink). The precipitate was allowed to settle overnight. The next morning the solution was filtered with Whatman No.40 filter paper. The precipitate was washed first with ammoniacal water and then with hot water several times until it was free from chloride. To test it 5 ml of the washing was collected, in a test tube and a drop of silver nitrate solution was added. The washing was continued till there was no precipitate with silver nitrate or calcium chloride solution. The filter paper was collected in a flask by making a hole in the filter paper. To this, 2 ml of 2N sulphuric acid was added. This solution was heated to 60-80°c and when still hot was titrated against N/100 potassium permanganate solution. From the volume of potassium permanganate solution used up the milligrams of calcium present in 100g of the sample was calculated.

f. Iron Estimation

The food sample is oxidized with ignition or oxidation. Iron as ferric iron reacts with ammonium thiocyanate or with potassium thiocyanate to give ferric thiocyanate which is red in colour. The colour which is a measure of the concentration is measured colorimetrically.

Took different aliquots of the standard solution (1 ml – 5ml) corresponding to 10-50 gamma in a series of test tubes. 2ml of the unknown solution was taken in the test tube. Added 1ml of 30per cent H_2SO_4, 1ml of potassium persulphate and 1.5ml of potassium thiocyanate to all the test tubes. This was made upto 10ml with water. A blank was prepared by adding the reagents except the standard or the unknown solution. Allowed the colour to develop for 20 minutes and the intensity was read at 530-540 μ filter in the colorimeter.

PLATE 16

CALCIUM ESTIMATION

PLATE 17

IRON ESTIMATION

g. Magnesium, Zinc and Copper Estimation

Minerals namely magnesium, zinc and copper were analysed 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. They were determined with a Buck atomic absorption spectrophotometer.

h. Selenium Estimation

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 Con 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 (Perkin Elmer model 3110) involving direct aspiration of the solution using graphite furnace (furnace Perkin Elmer HGA 600) (Andre 1992). Absorption of selenium was read at 196 nm with a slit width of 0.2nm (Raghuramulu *et al*., 2003)

Figure 3 gives the Research design followed in the present investigation.

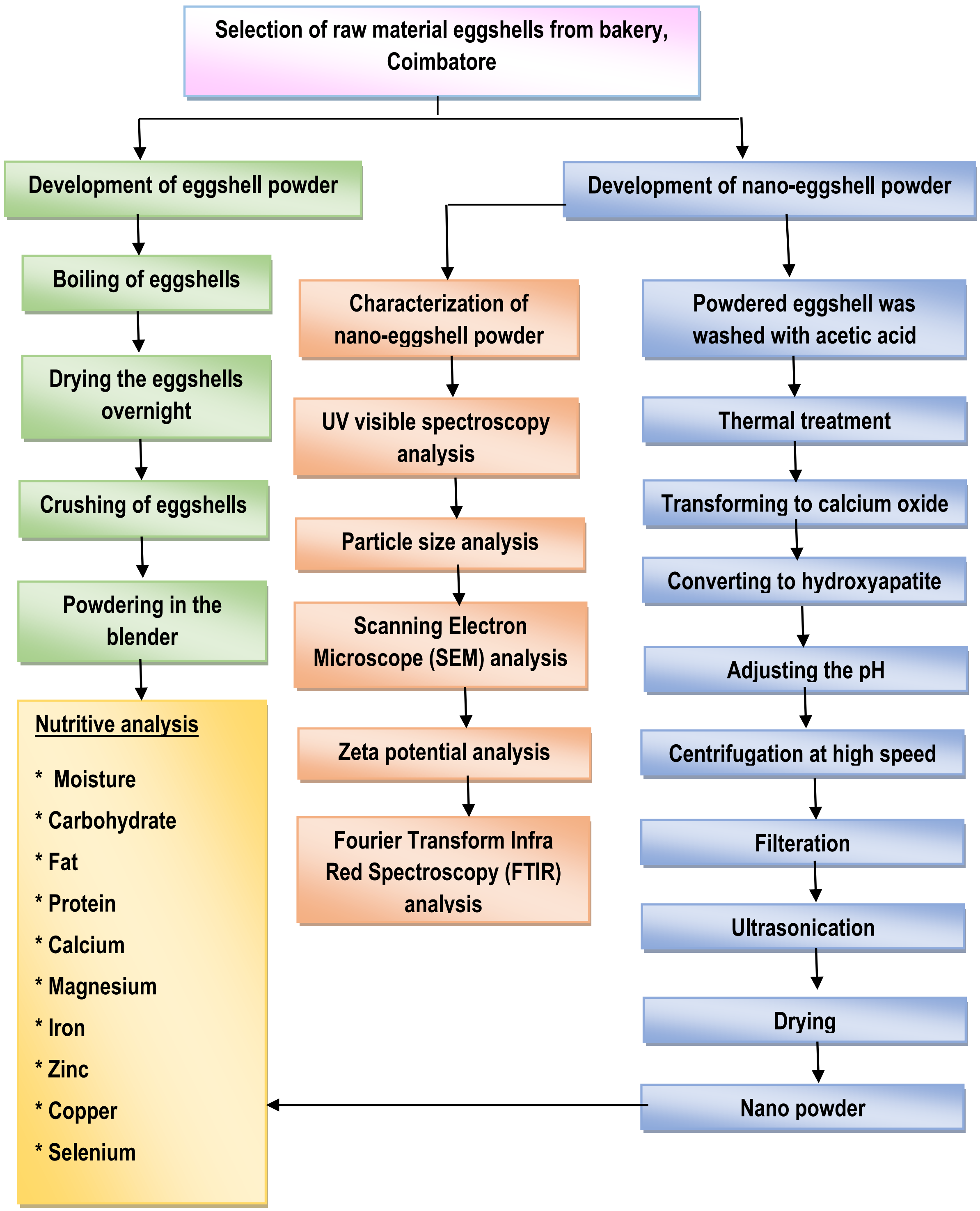


FIGURE 3

RESEARCH DESIGN

IV RESULTS AND DISCUSSION

The results of the study entitled **“Development and Nutritional Evaluation of Eggshell Powder and its Nanoparticles”** are presented and discussed under the following headings:

A. Development of Eggshell Powder
B. Characterization of Eggshell Nanoparticles
C. Macronutrient Content of Eggshell powder and its Nanoparticles
D. Micronutrient Content of Eggshell powder and its Nanoparticles
E. Comparison of Nutrient Content of Eggshell powder and its Nanoparticles

A. DEVELOPMENT OF EGGSHELL POWDER

Chicken eggshell is a waste material from domestic sources such as hatcheries, poultry farms, egg product factories, homes and restaurants. The eggshell powder was made from the fresh eggshells collected from bakery. The eggshells were crushed and then blended to make powder. A fine and white powder of eggshell was obtained.

Eggshell powder is a natural source of calcium and other elements (e.g. strontium and fluorine) which may have a positive effect on bone metabolism. Experimental and clinical studies performed by Rovensky *et al* (2003) have shown a number of positive properties of eggshell powder, such as antirachitic effects in rats and humans. A positive effect was observed on bone density in animal models of postmenopausal osteoporosis in ovariectomized female rats. *In vitro* eggshell powder stimulates chondrocyte differentiation and cartilage growth.

Clinical studies in postmenopausal women and women with senile osteoporosis showed that eggshell powder reduces pain and osteoresorption and increases mobility and bone density or arrests its loss. The bioavailability of calcium from this source, as tested in piglets, was similar or better than that of food grade purified calcium carbonate. Clinical and experimental studies showed that eggshell powder has positive effects on bone and cartilage and that it is suitable in the prevention and treatment of osteoporosis.The eggshell powder was used as a stabilizing material for improving soil properties (Amu *et al*., 2005), as a source of calcium for piglets (Schaafsma and Beelen, 1999) and as a source of calcium in human nutrition (Schaafsma *et al.*, 2000).

High-quality proteins make a valuable contribution to the synthesis and maintenance of muscle and indirectly to the regulation of blood glucose levels, thus contributing to power, strength, and energy. Eggs have traditionally been used as the standard of comparison for measuring protein quality because of their essential amino acid (EAA) profile and high digestibility. They provide a nutrient-dense source of energy from protein and fat, approximately 75 kcal per large egg, as well as several B vitamins, including thiamin, riboflavin, folate, B_{12}, and B_6, which are required for the production of energy by the body (Layman *et al*., 2009).

B. CHARACTERIZATION OF EGGSHELL NANOPARTICLES

The eggshell nanoparticles were prepared by the method of chemical precipitation via aqueous slurries. Applications of nanotechnology are being explored for use in foods and as ingredients. The available studies are limited but raise concerns about the absorption of nanoparticles in the gut and the potential impacts on nutritional status.

To understand the potential of nanoparticles, a deeper knowledge of their synthesis and applications is needed. The results of characterization using a variety of different techniques are presented below.

1. 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 of light it can absorb.

The Figure 4 represents the results of UV visible spectroscopy analysis on eggshell nanoparticles. The results showed a peak at 320 nm wavelength and then the absorbance sustained at the same level.

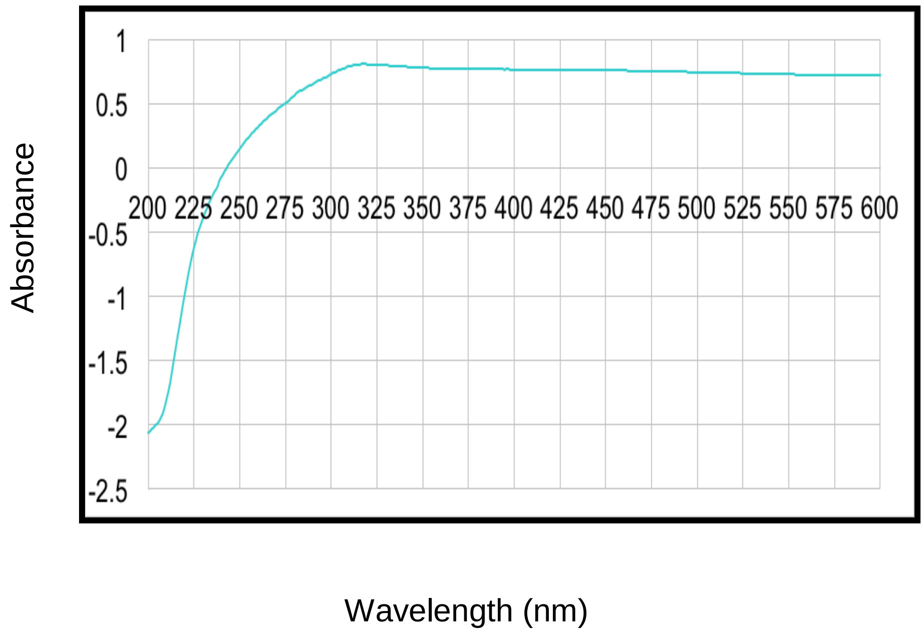


FIGURE 4

UV VISIBLE SPECTRA OF EGGSHELL NANOPARTICLES

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

The Figure 5 shows the particle size results of the nano eggshell particles. The mean, standard deviation and mode of nano eggshell particles are 79.1nm, 5.2nm and 78.5nm respectively, revealing that the eggshell nano particle has good surface area and absorbance.

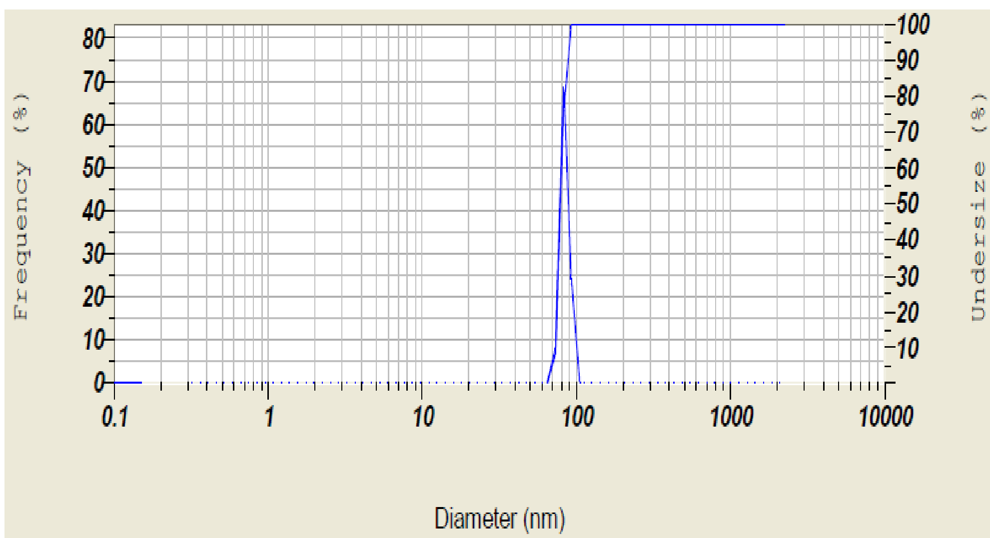


FIGURE 5

PARTICLE SIZE ANALYSIS OF EGGSHELL NANOPARTICLES

3. Scanning Electron Microscope (SEM) Analysis

The surface topography of nanoparticles was observed with a Scanning Electron Microscope (SEM). A scanning electron microscope 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 (Plate 18) shows the development of eggshell nanoparticles.

The SEM micrographs of nanoparticle 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 eggshell powder.

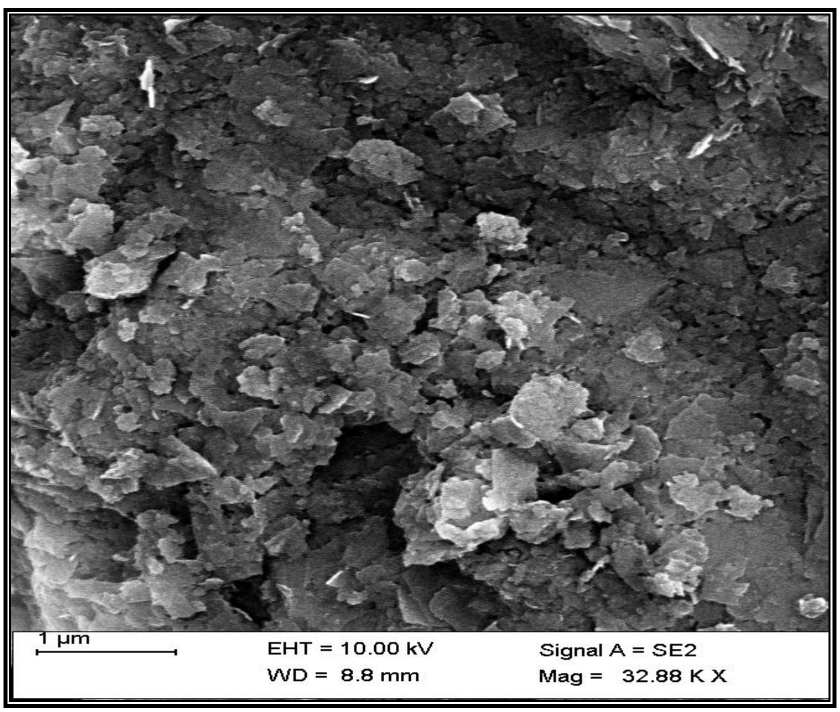


PLATE 18

SEM IMAGE OF EGGSHELL NANOPARTICLES

4. 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. The results from Figure 6 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.

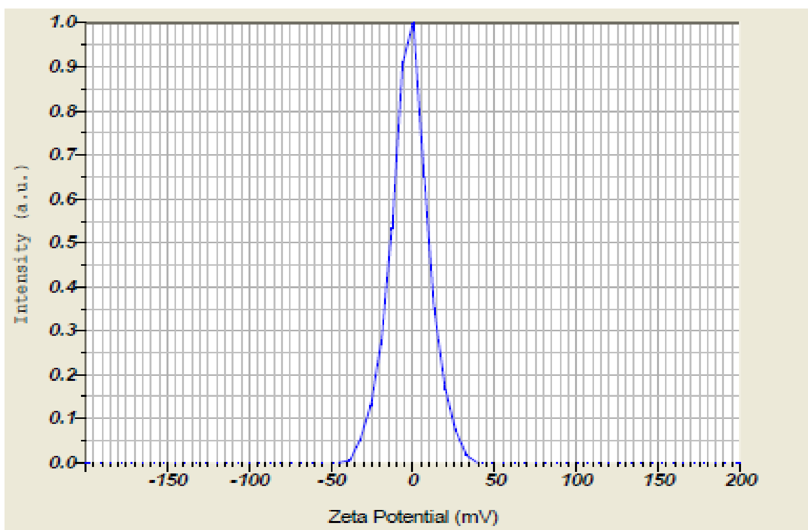


FIGURE 6

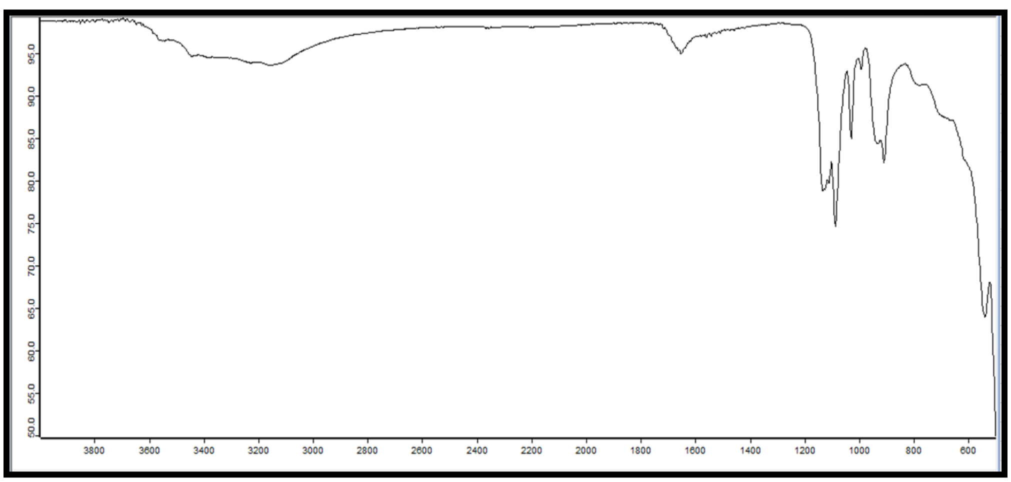
ZETA POTENTIAL ANALYSIS OF EGGSHELL NANOPARTICLES

5. Fourier Transform InfraRed Spectroscopy (FTIR) Analysis

In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample.

The results of the ultimate analysis of the Fourier Transform InfraRed Spectroscopy (FTIR) is shown in the figure 7. The most significant peak of intensity of nano eggshell particles at 1106cm-1, strongly associated with the presence of carbonate minerals within the eggshell matrix. There are also two observable peaks at about 1197cm-1 and 912cm-1, respectively, which may be associated with the presence of calcium carbonate.

Transmittance



Wavelength (cm-1)

FIGURE 7

FTIR SPECTRA OF EGGSHELL NANOPARTICLES

C. MACRONUTRIENT CONTENT OF EGGSHELL POWDER AND ITS NANOPARTICLES

Nutrients are substances needed for growth, metabolism, anfor other body functions. Macronutrients are required in large amounts that provide the energy needed to maintain body functions and carry out the activities of daily life. Table I shows the macronutrient composition of eggshell powder and its nanoparticles.

TABLE I

MACRONUTRIENT COMPOSITION

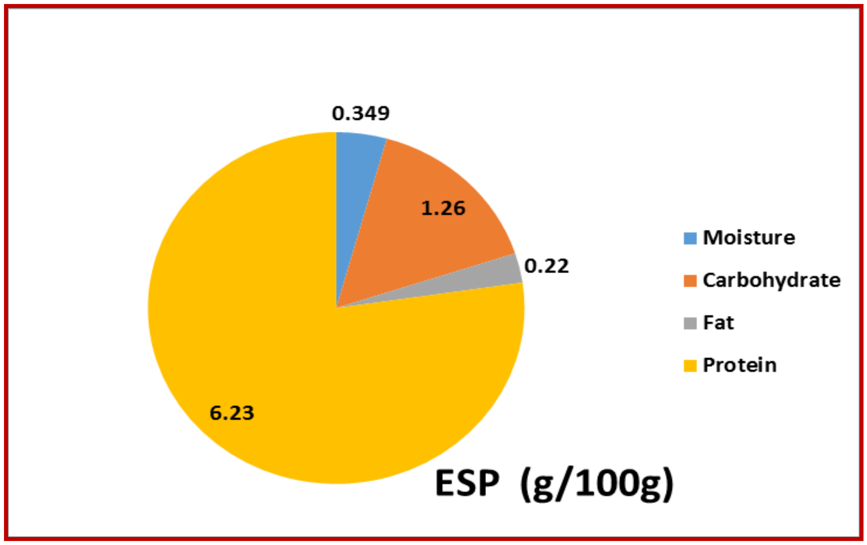
NUTRIENT	ESP (g/100g)	NESP (g/100g)
Moisture	0.349	0.26
Carbohydrate	1.26	1.12
Fat	0.22	0.17
Protein	6.23	7.0

ESP – Eggshell Powder   NESP – Nano Eggshell Powder

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

The amount of carbohydrate in hundred gram of eggshell and nano eggshell powder were 1.26g and 1.12g respectively. Carbohydrates provide 40 to 75 per cent of total energy intake, thus constituting the most important energy source in human diets. The carbohydrate content was found to be low in both eggshell powder and nano eggshell powder.

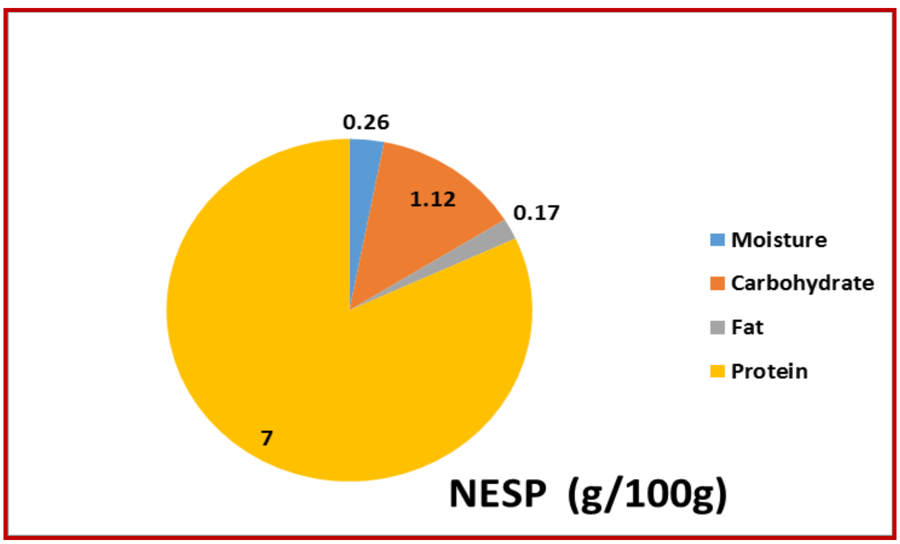
Figures 8 and 9 show the macronutrient contents of eggshell powder and its nanoparticles.



ESP – Eggshell Powder

FIGURE 8

MACRONUTRIENT CONTENT OF EGGSHELL POWDER AND ITS NANO PARTICLES



NESP – Nano Eggshell Powder

FIGURE 9

MACRONUTRIENT CONTENT OF NANO EGGSHELL POWDER

The FAO/WHO Expert Consultation on carbohydrates in human nutrition recommended an optimum carbohydrate level of at least 55 per cent of total energy intake, obtained from a variety of food sources, and noted that levels of carbohydrate consumption at or above 75 per cent of total energy should be avoided because of the exclusion of adequate amounts of protein, fat, and other essential nutrients. In 2000 Kcal diet 275 – 300g of carbohydrate is recommended (Gray, 2003).

The fat content was found to be 0.22g per 100g in eggshell powder and 0.17g per 100g in nano eggshell powder. Fat is a major source of energy and helps your body absorb vitamins. It is also important for proper growth, and for keeping you healthy. A completely fat-free diet would not be healthy, yet it is important that fat be consumed in moderation. It is important to keep in mind that fat has the most calories compared to any other nutrient. Controlling fat intake is one of the most important steps in losing or maintaining weight and preventing or delaying type 2 diabetes.

The protein content in eggshell and nano eggshell powder are 6.23g per 100g and 7.0g per 100g respectively. The amount of protein needed varies for different age groups, size and growth stage. Even though an adult has achieved maximum growth, protein is required for maintaining body tissues. Periods of growth, including infancy, childhood and pregnancy, increase the protein need to provide building materials. Physiological states such as injury, surgery, or burns, increase the need for protein to provide repairing materials (Lauritzen,1992). This reveals that the eggshell powder and its are rich in protein and can be supplemented according to the toxicity level of the powder.

Adults should get 45 per cent to 65 per cent of their calories from carbohydrates, 20 per cent to 35 per cent from fat, and 10 to 35 per cent from protein. Acceptable ranges for children are similar to those for adults, except that infants and younger children need a slightly higher proportion of fat (25 -40 per cent).

D. MICRONUTRIENT CONTENT OF EGGSHELL POWDER AND ITS NANOPARTICLES

Micronutrients are nutrients required by humans and other organisms throughout life in small quantities to orchestrate a range of physiological functions. For people, the dietary trace minerals in amounts generally less than 100 milligrams/day as opposed to macro minerals which are required in larger quantities. The micro minerals or trace elements include iron, cobalt, chromium, copper, iodine, manganese, selenium and zinc. Micronutrients also include vitamins, which are organic compounds required as nutrients in tiny amounts by an organism, as well as phytochemicals. Table II shows the micronutrient composition of eggshell powder and its nanoparticles.

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 – Eggshell Powder NESP – Nano Eggshell Powder

The eggshell powder contains 385mg/g of calcium and the nano eggshell powder contains 420mg/g. The Slovakian eggshell powder and chicken eggshell powder appear to have a comparable calcium composition (Schaafsma *et al*., 2000). The Slovakian eggshell powder was found have 386-415 mg of calcium in one gram. Figure 10 and 11 show the micronutrient content of eggshell powder and its nanoparticles.

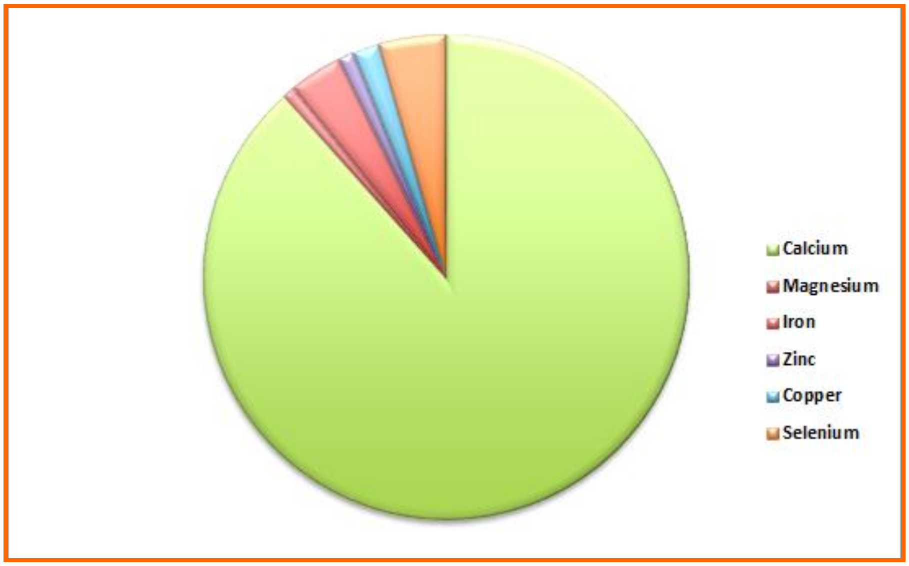


FIGURE 10

MICRONUTRIENT CONTENT OF EGGSHELL POWDER

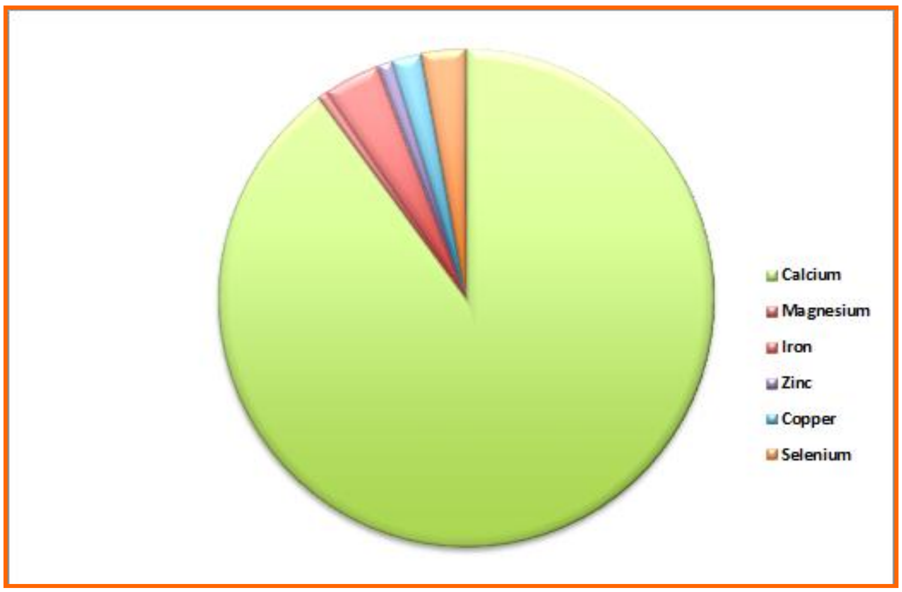


FIGURE 11

MICRONUTRIENT CONTENT OF NANO EGGSHELL POWDER

According to Pravina *et al* (2013), daily body requirement of calcium is about 450 mg which is nearly met by the nano eggshell powder. The adult requirements of calcium vary. During pregnancy and lactation, there is greater depletion of calcium from the mother and the intake needs to be increased. On an average, 10 mg per Kg of body weight per day should be sufficient. Growing children would require from 40 to 60 mg per day.

The amount of magnesium in one gram of eggshell and nano eggshell powder are 3.6mg and 3.2mg respectively. The Slovakian eggshell powder contains 3.5-5.5mg/g of magnesium (Schaafsma *et al*., 2000). From this data it is evident that the magnesium content is higher in Slovakian eggshell powder than that of chicken eggshell powder analysed in the present study.

The human body contains about 760 mg of magnesium at birth, approximately 5g at age 4–5 months, and 25 g when adult. Of the body's magnesium, 30–40 per cent is found in muscles and soft tissues, 1 per cent is found in extracellular fluid, and the remainder is in the skeleton, where it accounts for up to 1 per cent of bone ash. Between 50 per cent and 60 per cent of body magnesium is located within bone, where it is thought to form a surface constituent of the hydroxyapatite (calcium phosphate) mineral component. The acceptable range of intakes for adults of 150–500 mg/day (FAO., 2004). The results reveals that the amount of magnesium in eggshell and nano eggshell powder is lower than the dietary recommendation.

The iron content was found to be 15.3mg/g in eggshell powder and 17.1mg/g in nano eggshell powder. Schaafsma *et al* (2000) experimentally proven 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.

Nadadur et al., (2008), quoted that, iron is a micronutrient and its daily intake in milligram amounts is adequate for normal health. Despite such a low dietary requirement, iron deficiency due to malnutrition is a global health problem. An average adult human body has a typical iron content of approximately 4g and about 50 per cent of which is in haemoglobin, about 25 per cent is stored in liver and rest constitute myoglobin and numerous other iron containing proteins. Humans ingest approximately 12-18 mg/day of dietary iron, of which only 1-2 mg is absorbed. The recommended dietary allowance of iron for man is 17mg/day and female is 21mg/day which can be satisfied by the nano eggshell powder.

The zinc content in eggshell and nano eggshell powder are 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.

Zinc concentration of lean body mass is approximately 30 µg/g. Plasma zinc has a rapid turnover rate and it represents only about 0.l per cent of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye 274 µg/g and in prostatic fluids 300-500 mg/l. The recommended dietary allowance of zinc for man is 12mg/day and female is 10mg/day (FAO., 2004). Thus the zinc content is lower in both the eggshell powder and nano eggshell powder.

The proportion of copper in eggshell powder is 7.7mg/g and nano eggshell powder is 9mg/g. Srilakshmi.,(2008), reported that copper is necessary for the maintenance of the normal haemoglobin status and is also the part of many enzyme systems. The healthy human adult body contains about 100–150mg of copper. It plays an important role in the metabolism of fatty acids and in the formation of ribonucleic acid. Copper in the body is capable of binding bacterial toxins and increase the activity of antibiotics. The desirable intake suggested by ICMR of copper for an adult man is 2.2mg/day. This evidently show that the eggshell and nano eggshell powder are capable of providing even excess amount the nutrient which can be used in the case of deficiency.

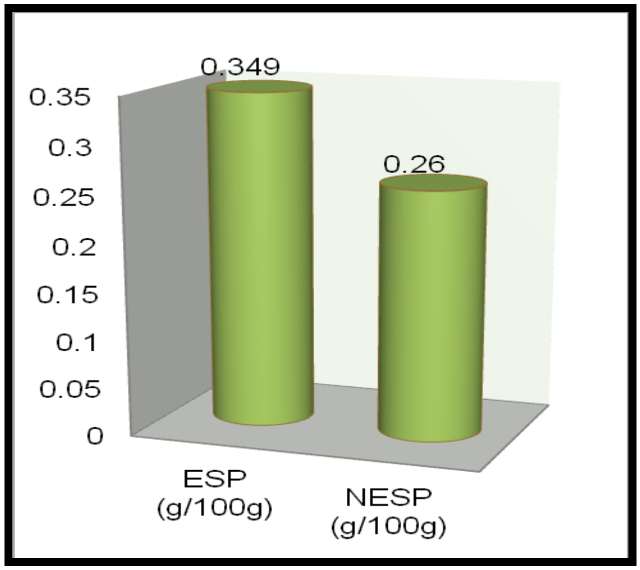
The selenium level in eggshell and nano eggshell powder are 19.4mg/g and 13.4mg/g respectively. FAO in 2004 suggested that selenium has been implicated in the protection of body tissues against oxidative stress, maintenance of defences against infection, and modulation of growth and development. Recommended dietary intake for infants is 14 μg/day and adults 48μg/day. This shows that when compared to other food groups selenium is rich in eggshell and nano eggshell powder.

E. COMPARISON OF NUTRIENT CONTENT OF EGGSHELL POWDER AND ITS NANOPARTICLES

The macronutrients were analysed for the eggshell and nano eggshell powder by experimental procedures, the amount of carbohydrate is higher in eggshell powder when compared to the nano eggshell powder. While the fat composition is lesser in the nano eggshell powder than the eggshell powder. Increased protein content is found in the nano eggshell powder than the eggshell powder.

The experimental procedures were done to analyse the micronutrient content in the eggshell powder and nano eggshell powder. The result shows that calcium is the major micronutrient present. The calcium content is higher in the nano eggshell powder than the eggshell powder. While the magnesium content is greater in the eggshell powder than the nano eggshell powder. When eggshell powder and nano eggshell powder were compared, iron content is excess in nano eggshell powder. The amount of zinc is little high in the nano eggshell powder than the eggshell powder. The copper level is more in the nano eggshell powder than the eggshell powder. The selenium level in the nano eggshell powder is less when compared to the eggshell powder. Figure 12 to 21 depict the nutrient composition of nanoparticles and ordinary eggshell powder on comparison.

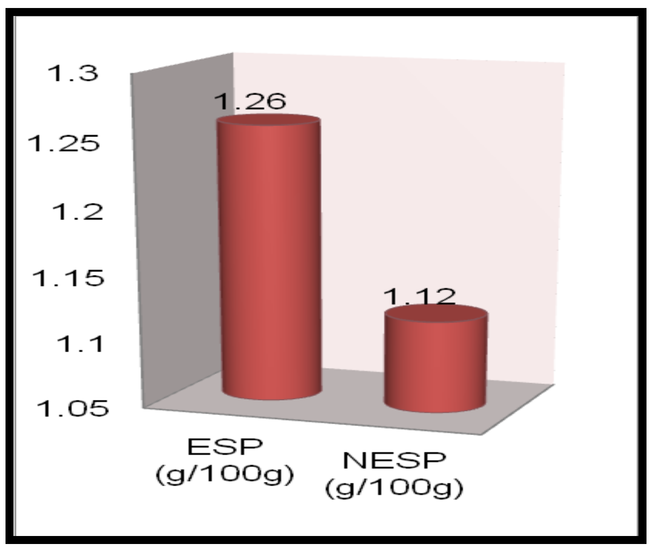
The nano eggshell powder with its high per centage of calcium, relevant amounts of magnesium, iron, zinc, copper and selenium should be a useful ingredient for human calcium enrichment strategies. Increases in bone mineral density and reduction of pain as reported from two small studies in subjects with osteoporosis support this application (Makai and Chudacek, 1991, Schaafsma and Pakan, 1999). Levels of microelements, such as magnesium, zinc and selenium in eggshell, can be improved and controlled via the feeding of the chickens (Lichtenstein, 1948). Therefore, a large store of wasted calcium may be a tailor made calcium source for postmenopausal women and the elderly population in particular.



ESP – Eggshell Powder NESP – Nano Eggshell Powder

FIGURE 12

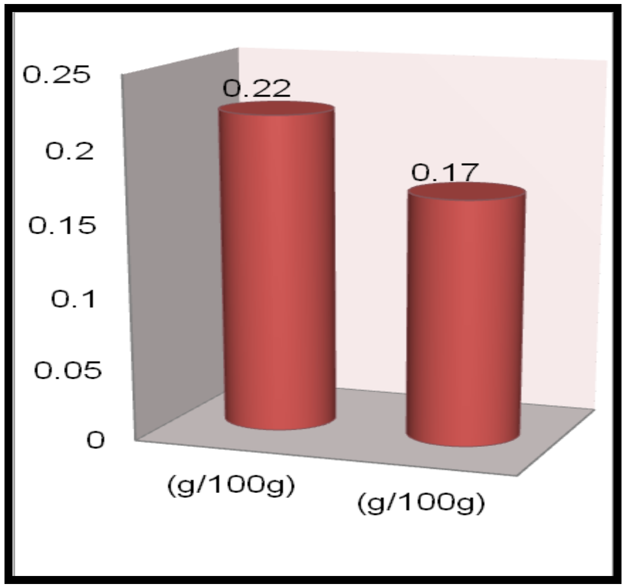
MOISTURE CONTENT



ESP – Eggshell Powder NESP – Nano Eggshell Powder

FIGURE 13

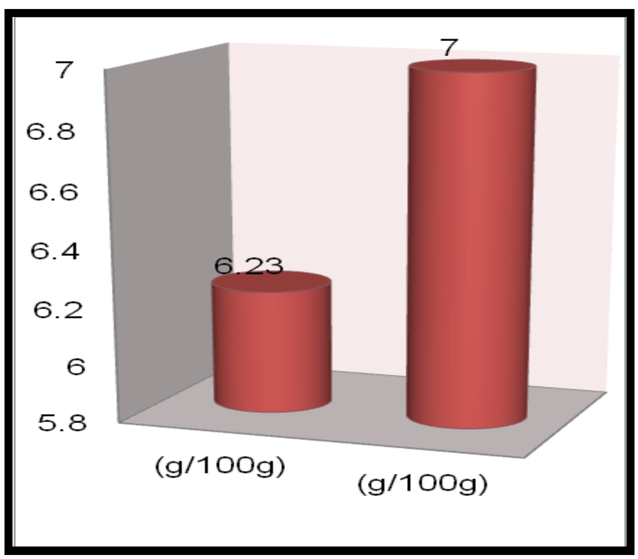
CARBOHYDRATE CONTENT



ESP – Eggshell Powder NESP – Nano Eggshell Powder

FIGURE 14

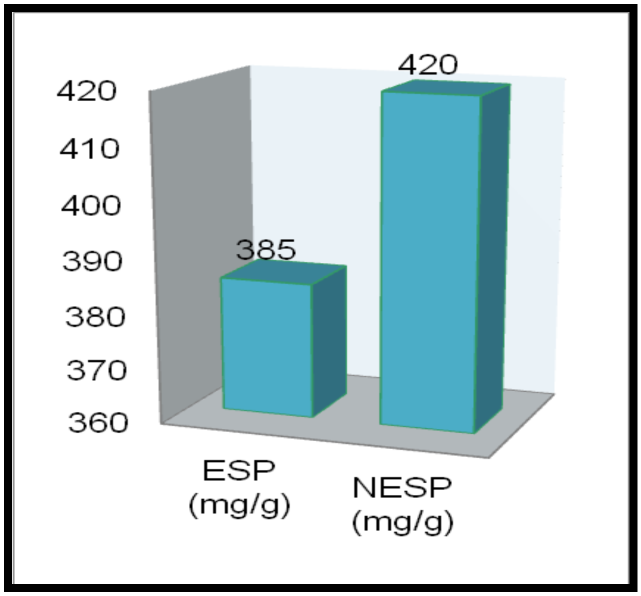
FAT CONTENT



ESP – Eggshell Powder NESP – Nano Eggshell Powder

FIGURE 15

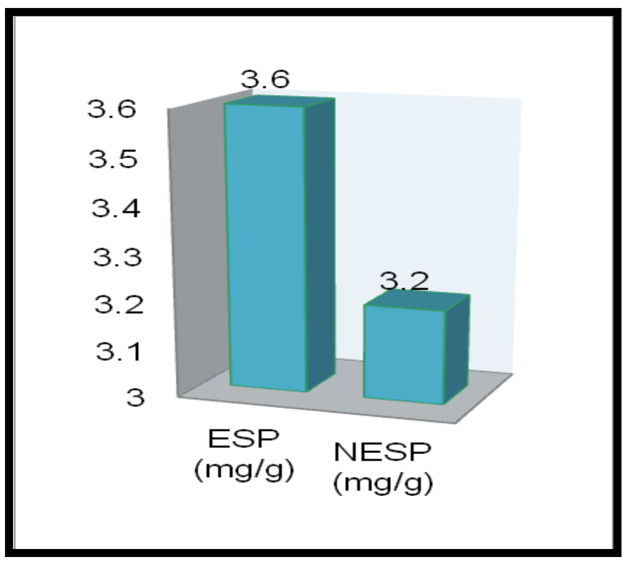
PROTEIN CONTENT



ESP – Eggshell Powder NESP – Nano Eggshell Powder

FIGURE 16

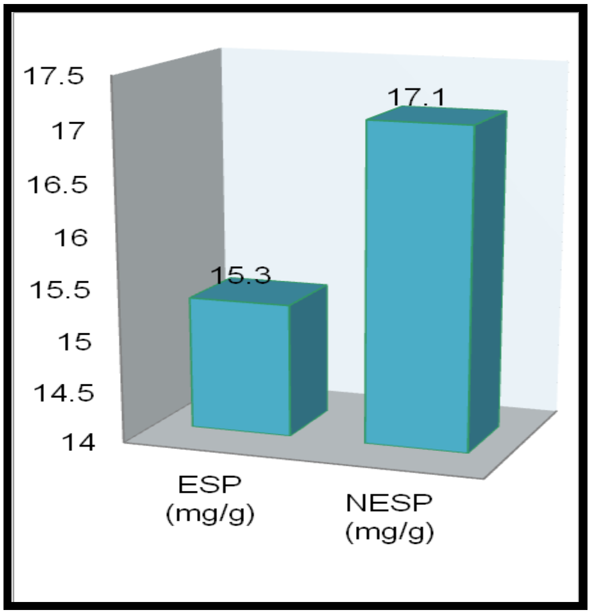
CALCIUM CONTENT



ESP – Eggshell Powder NESP – Nano Eggshell Powder

FIGURE 17

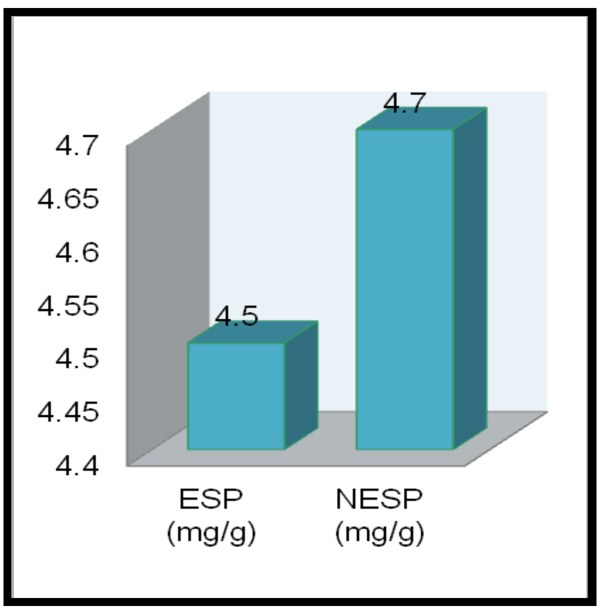
MAGNESIUM CONTENT



ESP – Eggshell Powder **NESP – Nano Eggshell Powder**

FIGURE 18

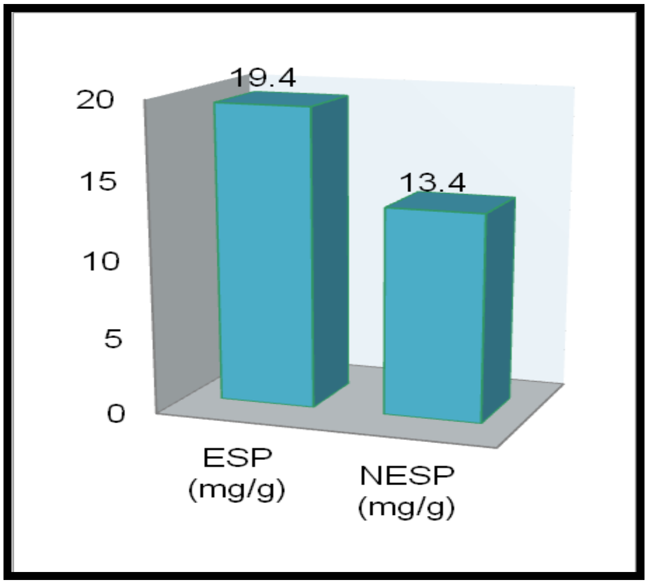
IRON CONTENT



ESP – Eggshell Powder **NESP – Nano Eggshell Powder**

FIGURE 19

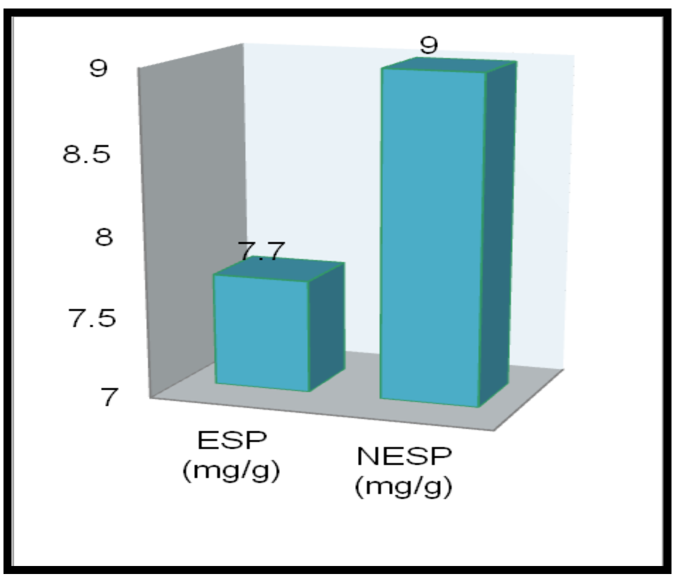
ZINC CONTENT



ESP – Eggshell Powder NESP – Nano Eggshell Powder

FIGURE 20

COPPER CONTENT



ESP – Eggshell Powder NESP – Nano Eggshell Powder

FIGURE 21

SELENIUM CONTENT

V SUMMARY AND CONCLUSION

Agricultural waste utilization has made contributions in many aspects of life from its use as feedstock in the production of biobased products and its subsequent use as source of renewable energy. Its utilization has the potential of reducing pollution level and environmental effect exerts by dependence on chemical products. Its use as a biomaterial in biomedical applications has added more value to its utilization. Eggshell produced by chickens is considered as useless mostly but consequently it has value in biomedical applications. Eggshell is considered to be a rich source for calcium as carbonates and oxides that qualify the most excellent sources of hydroxyapatite.

Although eggshells are not the first natural source for the synthesis of hydroxyapatite, it provides a cost effective and a renewable as well as a sustainable source of biological value. With this development, eggshell agricultural waste has made a significant contribution in the production of biomaterials used in medical applications. Hydroxyapatite is an excellent material used in bone repair and tissue regeneration. In India, eggshell powder has been used in manure development by different companies. Globally a few studies are available in animals especially in osteoporosis by supplementation of eggshell powder. Since studies on humans are not available, it was thought of interest to conduct the present study.

With this background, the present study entitled **"Development and Nutritional Evaluation of Eggshell Powder and its Nanoparticles"** was undertaken. The objectives of the study were to develop eggshell powder and its nanoparticles, evaluate the nutritive value of eggshell powder and its nanoparticles and comparing the nutritive value. The uncrushed eggshell was collected and boiled. The boiled eggshell was dried overnight and then powdered. The powdered eggshell was taken for further evaluation of nutrient content. The nanoparticles were prepared by chemical precipitation via aqueous slurries comprising of biowaste eggshells. The powdered eggshell were washed with acetic acid and placed for thermal treatment. This transforms the eggshell into calcium oxide. The transformed calcium oxide from heat treated eggshells is converted into hydroxyapatite with phosphate solution. The pH of the solution were adjusted and then kept for incubation. After incubation, the slurry was centrifuged and sludge was filtered. After filtration, the precipitate was dissolved in ethanol and then ultrasonication is done which is followed by drying. Thus, the nanoparticles of eggshell were recovered. Nearly 500g of the uncrushed eggshell was taken and they were converted to nanoparticles weighing 30g. The process of transforming eggshell into nanoparticles is an environmental friendly process.

The eggshell nanoparticles were characterized for the better knowledge of the size, shape, stability, absorbance and transmission. Analysis of UV visible spectroscopy, particle size, Scanning Electron Microscope (SEM), zeta potential and Fourier Transform InfraRed Spectroscopy (FTIR) were undergone for the eggshell nanoparticles. The nutritive analysis of the eggshell powder and its nanoparticles were evaluated for moisture, carbohydrate, fat, protein, calcium, magnesium, iron, zinc, copper and selenium using NIN manual procedures *(*Raghuramulu *et al*., 2003*)*. Eggshell nanoparticles may stand as good chance of reducing the cost of treatment in bone repair or replacement with little impact on the environment.

The salient findings of the study are:

- The eggshell powder was developed from the fresh eggshells. The powder was made in the blender. The obtained powder of eggshell were fine in texture and white in color. The eggshell development process was feasible and environmental friendly.
- The nanoparticles of eggshell powder when subjected to characterization showed an absorbance of energy from ultra- violet rays were obtained from UV visible spectroscopy analysis. The result

of the UV visible spectroscopy analysis shows that the absorbance level sustain after 320nm of wavelength.

- The particle size analysis of the nanoparticles were done for the better knowledge of the morphology of the eggshell nanoparticles.the results of the analysis reveals that the eggshell nanoparticles revealed that the nanoparticles have good surface area and absorbance level.
- The Scanning Electron Microscope (SEM) analysis reveals that the developed nanoparticles of eggshell confirmed the nanostructures of eggshell. The micrographs of nanoparticle obtained in the filtrate showed that they are round shape and the particles are uniformly distributed over the surface.
- The zeta potential analysis were performed to know the stability level of the eggshell nanoparticles. From the results, it is evident that the eggshell nanoparticles is less in stability as the value is obtained in negative range.
- The Fourier Transform InfraRed Spectroscopy (FTIR) analysis reveals the most significant peak of intensity of nano eggshell particles at 1106cm-1, strongly associated with the presence of carbonate minerals within the eggshell matrix.
- The moisture content of the eggshell powder was found to be 0.349g per 100g while the eggshell nanoparticles contains 0.26g per 100g. The amount of carbohydrate content in 100g of the eggshell powder was found to be 1.26g while the eggshell nanoparticles contains 1.12g.
- The fat content of the eggshell powder was found to be 0.22g per 100g while the eggshell nanoparticles contains 0.17g per 100g. The amount of protein content in 100g of the eggshell powder was found to be 6.23g while the eggshell nanoparticles contains 7g.

- The calcium content of the eggshell powder was found to be 385 mg per gram while the eggshell nanoparticles contains 420 mg per gram. The amount of magnesium content in one gram of the eggshell powder was found to be 3.6 mg while the eggshell nanoparticles contains 3.2 mg.
- The iron content of the eggshell powder was found to be 15.3 mg per gram while the eggshell nanoparticles contains 17.1 mg per gram. The amount of zinc content in one gram of the eggshell powder was found to be 4.5 mg while the eggshell nanoparticles contains 4.7 mg
- The copper content of the eggshell powder was found to be 7.7 mg per gram while the eggshell nanoparticles contains 9.0 mg per gram. The amount of selenium content in one gram of the eggshell powder was found to be 19.4 mg while the eggshell nanoparticles contains 13.4 mg.

From the foregoing results, it is evident that the development of eggshell powder and eggshell nanoparticles were economical by normal blending method and chemical precipitation method respectively. Based on the characterization of the eggshell nanoparticles, it was interesting to note that the eggshell nanoparticles were in the size of 79.1nm, as evident from the particle size analyser. Scanning Electron Microscope (SEM) images showed more exciting 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 InfraRed 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 UV visible spectroscopy analysis shows that the peak raises at 320 nm of wavelength where the absorbance sustains in the same level.

In terms of nutrient potentials, the study revealed the eggshell powder and the nano eggshell powder appear to have a comparable macronutrient and micronutrient composition. However, the nano eggshell powder contains more calcium than the eggshell powder. Eggshell powder had lower levels of iron, zinc and copper than the nano eggshell powder. Hence, the present study proved the feasibility of preparing the eggshell powder and eggshell nanoparticles.

Recommendations for future research are:

- Studies on sub- chronic toxicity in animals and histopathology.
- Studies on osteoporosis in animals and intervention using eggshell powder.
- Testing eggshell nanoparticles in *in vitro* and *in vivo* conditions on toxicity.
- Studies on bioavailability of calcium from eggshell powder and its nanoparticles.
- Quantitative assay of total polyphenols and individual polyphenols.
- Impact of supplementation of eggshell powder and eggshell nanoparticles in animal models and humans on assessment of bone mineral intensity and relief of post menopausal symptoms.
- Development of dietary supplement or nutraceutical formulation using eggshell powder.

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