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EGG SHELL: AN ESSENTIAL WASTE PRODUCT TO IMPROVE DIETARY CALCIUM UPTAKE

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ABSTRACT

Since the human skeletal system is composed primarily of calcium (98%), calcium is one of the most fundamentally important and necessary elements for the human body. The current review paper aimed to address egg shell (ES) deposition, its nutritional composition, theories of calcium absorption, and the significance of calcium in daily life. ES formation occurs in two stages. Calcium carbonate, weighing 5.5 grams, makes up approximately 95% of the dried ES. Approximately 0.3% of phosphorus, 0.3% of magnesium, and traces of sodium, potassium, zinc, manganese, iron, and copper can all be found in one ES. Calcium absorption depends on vitamin D, therefore dietary intake and vitamin D status both affect how much calcium is bioavailable. Numerous locations along the nephron may be sites of calcium reabsorption. Some factors have been demonstrated to positively increase calcium absorption, including intestinal acidity (especially for $CaCO_3$ absorption), oestrogen, vitamin D, and soluble fiber/prebiotics, probiotics, and synbiotics. Studies have been done on pertinent processes that can be utilized to extract calcium from ESs, including pulsed electric fields, high-energy milling, and electric discharge-assisted mechanical milling. The study focused on the nutritional benefits of eggshells as well as several eggshell extraction techniques to determine their economic viability in producing a sufficient calcium supplement.

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Introduction

Calcium is one of the most fundamentally important and vital minerals for the human body, as it makes up 98 % of the skeletal system [1] dietary sources are used to supply this. It serves as a critical component of our bodies' structure, and hence its function is clear. Furthermore, data suggest that the consumption of dietary calcium in India has been steadily falling for the past half-century [2]. Calcium is a vital micronutrient that is necessary for tooth and bone growth, as well as muscular contraction, blood clotting, and nerve conductivity [3]. Calcium plays a crucial role in cardiovascular health, and getting enough of it is recommended to avoid hypertension and preeclampsia [4]. As the years pass, people's attention to getting enough calcium is waning, perhaps leading to poorer bone density and disorders like osteoporosis and hypocalcemia [2]. Osteoporosis is characterized by a gradual loss of bone density [5].

ESs are a significant source of calcium; therefore, research has explored new methods to include them in the human diet to meet their calcium needs. According to some clinical research, providing calcium from ESs to some organisms resulted in enhanced bone mineral density and an anthracitic effect. Enhanced bone strength, reduced pain feeling, and increased mobility is all signs of osteoporosis. ES calcium has a high bioavailability and has been shown to help with fragile fingernails, hair, lethargy, and asthma in both kids and young adults [6-9]. The present study looked into the nutritional profile of ES importance of calcium in the diet as well as the ingredients which are needed as support for calcium absorption.

Egg Shell (ES)

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ES serves primarily as a physical barrier to prevent bacteria from entering cells while yet allowing for gas exchange [10]. ESs from chickens is a waste product. They are a good source of calcium in the diet and can be used in place of crustacean shells [11]. Two stages of ES deposition take place. The longest stage of egg development lasts for roughly 17 hours in chicken breeds that are heavily selected as layers. The *first stage*, which lasts for around 5 hours, marks the beginning of mineralization. At the locations of the organic aggregates found on the surface of outer shell membranes, the first calcite crystals form. Although a keratan sulphate proteoglycan has been suggested, the makeup of these aggregates is unknown. These nucleation sites' distribution is determined genetically and differs between species [12-14]. The distance between these sites determines the size of the mammillary cones, the cylindrical diameter of the palisades in the compact layer of the shell, and ultimately the strength of the shell. Radial calcite is the crystal type that grows at the initial stage of mineralization. The sources of the mammillary cones are the nucleation sites [15]. Radial crystal growth is halted by mutual exclusion when they gradually converge to create the bases of the palisade layer as they spread outward. The rapid growth of polycrystalline calcite to create the palisade layer is the *second stage* [16]. The exterior ES and cuticle are concentrated with ovocalyxin-32, a significant phosphoprotein of the ES matrix, which makes it a possible contender as a proteinaceous crystal growth inhibitor [17-20].

Nutritional Profile of ESs

Calcified ES and shell membrane make up the chicken ES. Compared to the weight of the whole egg, its total weight is 10–11% less. The ES is composed of 98 % dry matter and just 2 % water when it comes to its chemical composition. On the other hand, ash makes up 93 % of the dry matter, whereas crude protein makes up 5 %. The membrane of the egg is a fibrous structure. It is crucial for the development of ES [21]. Chemically speaking, an ES from a hen has 65.6 percent water, 11.8 % proteins, 11 % fat, and 11.7 % ash. Chemically, ES powder is made up of 21.2 % carbon, 0.93 % magnesium oxide, 76.9 % calcium oxide, 0.42 % porosity, 0.02 % iron oxide, and 0.11 percent sodium oxide [22-25] **(Figure 1)**. ESs also contains 0.3 % phosphorus, which is found in little amounts but is particularly beneficial for regenerating hen bones. Additionally, hen ES contains 0.2 % magnesium, which has an impact on maintaining the quality of the ES. Calcium fulfillment in the shell ranges from 28.2 to 41.2 %, and phosphorus fulfillment is 0.102 percent [25].

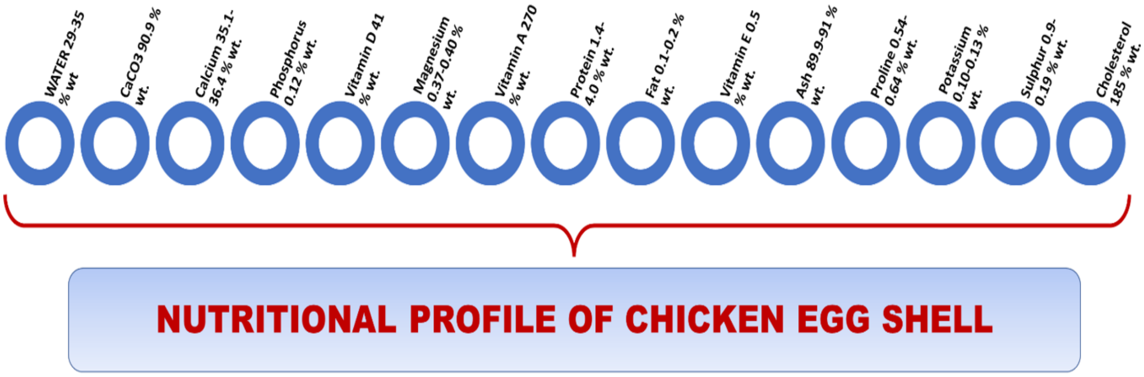

Figure 1. Nutritional values present in ES

Theory of Calcium Absorption

Vitamin D is necessary for calcium absorption; hence bioavailability is influenced by vitamin D status and dietary consumption. The effectiveness of absorption is dose-dependent and related to physiological calcium requirements [26]. On the other hand, lactose promotes the absorption of calcium. Both a passive, paracellular process and an active, carrier-dependent process are involved in calcium absorption. While the passive process is not dependent on vitamin D, the active process is. Low calcium intake leads to the conversion of 25(OH)D to 1,25(OH)2D, which increases the transcription of the calcium transport proteins in the gut. This homeostatic control mechanism, however, is unable to compensate for persistently low calcium intakes. When vitamin D reserves are too low, there is a decreased conversion to 1,25(OH)2D [27-30]. Another approach is net calcium absorption, which is based on metabolic balance and requires strict dietary restrictions as well as thorough urine and feces collections. The endogenous secretion, or ingested calcium that is re-excreted in the gut, is not corrected by this approach. Changes in serum calcium levels or Ipth (Parathyroid hormone) following a calcium challenge are indirect ways of calcium absorption **(Figure 2)**.

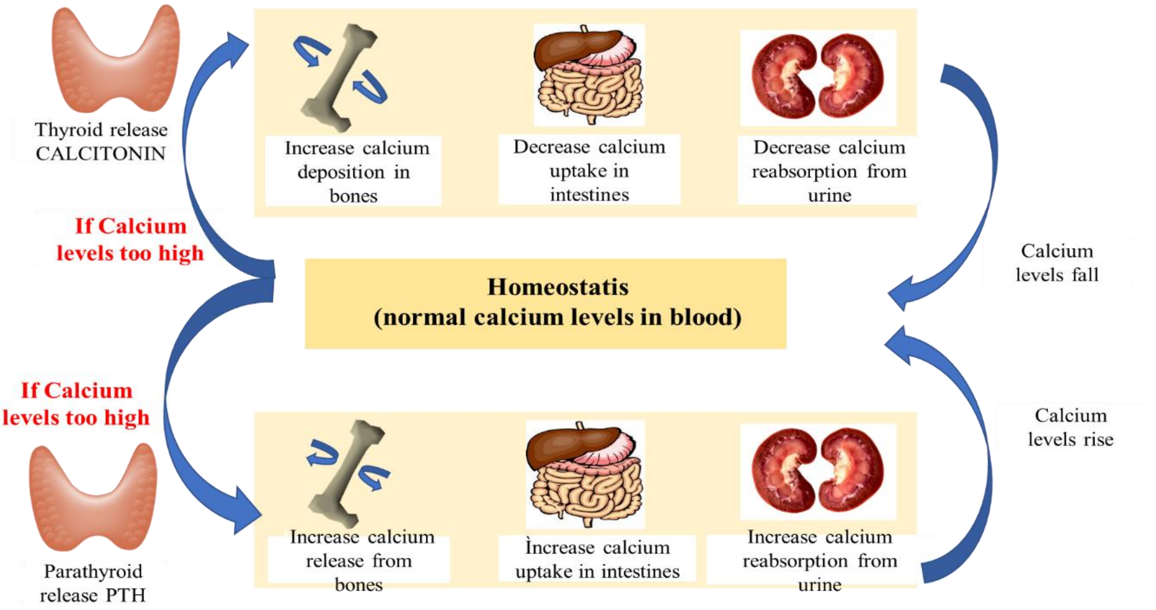

Figure 2. Figure showing homeostasis of calcium absorption

Both saturable (likely transcellular) and nonsaturable (likely paracellular diffusion) pathways are used for calcium to pass the intestinal barrier [23]. Calcium absorption is saturable in the duodenum, the small intestine's proximal segment, and to a lesser extent in the jejunum (midportion of the small intestine).

The uptake of calcium is also aided by *controlled paracellular transport*. The tight junction proteins claudin-2 and claudin-12 are produced in greater quantities by 1,25(OH)2D in the jejunum and ileum, enhancing passive transport [24]. Contrary to the ileum, where claudin-2 and claudin-12 expression is strongest, the proximal duodenum and jejunum are where vitamin D governs active calcium absorption. During pregnancy and lactation, prolactin upregulates claudin-15, which aids in paracellular calcium absorption. It also upregulates transcellular calcium transport via TRPV5, TRPV6, (transient receptor potential cation channel subfamily V member) and calbindin-D9K. The voltage-dependent L-type calcium channel subunit alpha-1D also referred to as voltage-gated calcium channel subunit alpha Cav1.3 may also aid in the absorption of calcium from the intestine. However, the gene for this protein is not controlled by vitamin D, and animals lacking Cav1.3 do not exhibit significant changes in either calcium or bone metabolism [25-31].

Intestinal calcium transport pathways and the timing of responses to 1,25(OH)2D cannot be entirely explained by a single model. Data support both transcriptional and faster increases in calcium transport in response to 1,25(OH)2D; faster increases in calcium transport imply that vesicular transport mechanisms may be significant. Given that relative dietary insufficiency is prevalent and excessive calcium consumption can also occur, redundancy in this system probably permits higher control and efficiency of calcium absorption [23, 27].

Renal Calcium Reabsorption

The nephron is the kidney's fundamental unit of functional organization. The glomerulus, the first nephron segment, filters blood. The filtered fluid and its contents then go via the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, and the connecting tubule along the nephron's course. Calcium reabsorption may take place at several places along the nephron. About 45% of the total calcium in the plasma is ionized and enters the glomerular filtrate. In the proximal convoluted tubule, calcium is passively and paracellularly reabsorbed to a degree of about 65 percent. Calcium reabsorption occurs in the proximal tubule as a result of solvent drag, whereas calcium transport is predominantly caused by calcium after the reabsorption of water rather than by ion channel action [27]. About 20–25 percent of the filtered calcium is reabsorbed in the thick ascending limb of the Henle loop. The sodium-(potassium)-chloride cotransporter 2 (NKCC2), ATP-sensitive renal outer medullary potassium channel (ROMK), and the basolateral sodium/potassium-transporting ATPase (NaK-ATPase) work together to maintain a positive electrochemical potential in the lumen on the apical (luminal) surface [28, 29]. This potential promotes the reabsorption of sodium, calcium, and magnesium cations paracellularly. The G protein-coupled extracellular calcium-sensing receptor (CaSR), encoded by the CaSR gene) detects excessive extracellular calcium on the basolateral (interstitial) side of the renal tubular epithelial cells in the thick ascending limb. Lowered ROMK expression is a result of high calcium.

Factors Supporting Calcium Absorption

Vitamin D

The limited dietary sources of vitamin D, decreased sun exposure, decreased skin's ability to synthesize vitamin D, and decreased kidney's ability to convert vitamin D into its active form put elderly persons at significant risk of vitamin D deficiency. The two main types of vitamin D are: The first is *vitamin D2 (ergocalciferol)*, which is produced when ergosterol, a naturally occurring vitamin D precursor present in plants, fungi, and invertebrates, is exposed to UV-B radiation. The second is *cholecalciferol, or vitamin D3*, which is produced when skin is exposed to sunlight and is derived from 7-dehydrocholesterol, a precursor to cholesterol that can also function as provitamin D3.

Prebiotics, Probiotics, and Synbiotics

Nondigestible fibers known as prebiotics serve as the substrates for gut microorganisms to process nutrients and energy sources. Prebiotics can also increase the survivability of gut microbes. Prebiotics has been shown in numerous trials to increase calcium absorption, which may lower the risk of osteoporosis. Prebiotics that contain fructans, such as inulin, oligofructose, and fructooligosaccharide (with a dosage of 4 to 8 g/day), improve calcium absorption. These fructans are fermented by gut microorganisms, and the result lowers the pH in the colon. This can boost the absorption of micro minerals, such as calcium, because calcium is converted to an ionic form in an acidic environment, making it more soluble and accessible. The short-chain fatty acids produced during the prebiotic fiber fermentation process, along with the acidic environment they create, cause the mucosal cells to swell, which increases the surface area of the intestine and enhances calcium absorption [18-25].

Fat

The bioavailability of fat-soluble vitamin D, which is essential for calcium absorption, is influenced by fat. The effect of fat on calcium absorption directly depends on the kind of fatty acids it produces [17]. Saturated fatty acids were added to the diet, which resulted in the most efficient absorption. Although saturated fatty acids help the body absorb calcium, their impact on bone health is smaller than that of a diet with a normal fat content or a similar proportion of monounsaturated fatty acids. Researchers discovered that mice given food enriched with monounsaturated fatty acids had considerably higher levels of hepatic cytochrome P450 protein, intestinal calbindine D9k gene expression, and trabecular volume thickness than mice given an unaltered diet. Therefore, a diet high in monounsaturated fatty acids is advised since it has the best effects on bone health and slightly increases intestinal calcium absorption [22].

Importance of Calcium in Daily Life

Bone Health

An asymptomatic loss of bone mass per unit volume is the hallmark of osteoporosis, a complex illness with numerous causes [26]. When a bone mass is too low, structural integrity and mechanical support are compromised, and fractures can result from even minor trauma [27-29]. Ribs, hummers, pelvis, distal radius, proximal femur, and distal radius fractures are more frequent. Elderly people and postmenopausal white women are more likely to sustain fractures [30]. Bone is a structure that is always being rebuilt or reconditioned and is metabolically active. Bone resorption cells and bone production cells control this function. In a typical, healthy adult bone, bone growth balances out bone resorption, leaving no overall difference [21]. Magnesium, phosphorus, and calcium, the main mineral ions of bones, are crucial in affecting variations in bone mass. For correct bone calcification and growth to take place, these mineral ions must be present in extracellular fluids at normal and sufficient levels [22].

Hypertension

Elderly people frequently experience hypertension and osteoarthritis, both of which are believed to share the same etiology-calcium deficiency [23]. A higher calcium intake is believed to be connected to a lower risk of cardiovascular disease, particularly in postmenopausal women. Calcium is known to play a significant role in the management of hypertension [24]. Calcium helps to regulate blood pressure as well, and some evidence indicates that people or lab animals with adequate calcium levels may be protected from hypertension. Pre-eclampsia and pregnancy-induced hypertension were significantly decreased when calcium intake was increased (1-2 g/day). Systolic and diastolic blood pressure has been demonstrated to decrease with calcium supplementation in the diet; however, people with low calcium intake (less than 0.8 g/day) had higher blood pressure. There needs to be more investigation into how calcium affects systolic and diastolic blood pressure [25]. According to a meta-analysis, calcium supplementation during pregnancy among women at risk of calcium deficiency lowers the incidence of pre-eclampsia by more than 50%. ES calcium has a high bioavailability, therefore even though there isn't enough data to support its use in the treatment of calcium, we can anticipate good results.

Weight Loss

Calcium may have a function in controlling body weight based on observations showing that eating a meal high in calcium during times of high energy consumption lowered adipocyte lipid deposition and weight gain. Through parathyroid hormone, calcium-rich diets lower intracellular calcium ions [27]. The increased intracellular calcium would result in a decrease in fatty acid synthase expression. This enzyme is essential for regulating lipid deposition. Higher weight loss is ascribed to lipids while also promoting fat tissue breakdown as a result of the accelerated oxidation following calcium. Additionally, dietary calcium encourages the excretion of fecal fat, leading to an increase in fecal fat excretion. The mineral hydroxyapatite, which is present in fish bones and marine shells, can also be taken as a supplement [28-30]. People who consume a lot of calcium have higher levels of the plasma peptide tyrosine, which improves satiety and reduces calorie intake, promoting weight loss [30].

Colorectal Cancers

The biggest cause of death in the West is colorectal cancer. It has long been believed that dietary calcium can help prevent adenomas and colorectal cancer. It has been demonstrated that calcium lowers the risk of colorectal cancer via binding to ionized fatty acids and carcinogenic bile acids. This made it harder for these cancer-causing substances to flourish in the mucosal layer of the colon. By binding to calcium receptors, calcium is also known to support the diversity of selectivity of colon cells. The activation of isozyme protein kinase C is just one of the biological effects of this. Colon cancer cells have been found to express this protein differently in both humans and animals. Numerous clinical investigations have demonstrated that calcium supplements can aid in defending colon cells against pre-malignant changes. The use of calcium supplements and the emergence of distal colon cancer seem to be related [15-19].

Calcium Signaling

To adapt to shifting circumstances, cells must signal, and this communication requires messengers whose concentrations change over time. A cell's life and death are affected by calcium ions in every way. Numerous proteins have interactions with calcium ions that alter their behavior, associations, and localization. Almost all cell types, including immune system-related T-lymphocytes, B-lymphocytes, and mast cells, depend on calcium as a universal dispatch rider. Additionally, the body uses calcium signaling in a variety of processes, including growth and proliferation, specification, and programmed cell death (apoptosis) (mRNA), to replicate genetic material (DNA) into new molecules [26].

General Methods of Calcium Extraction

ESs are one of the most widely distributed biomaterials in nature. Since they are discarded following the use of egg yolk and albumin, they represent a significant waste produced by the food industry [28].

Pulsed Electric Field (PEF)

Due to its low cost, the novel extraction method known as the pulsed electric field has recently attracted a lot of attention in the food and pharmaceutical industries [14]. Initially, this method was employed as a non-thermal method to inactivate bacteria and enzymes at room temperature to enhance the value of food and medicinal materials (i.e., it does not raise the temperature of the food during processing) [23, 24]. PEF can also be used to extract and recover high-value compounds from food molecules, inactivate food item enzymes, and increase food stability and safety. This method also modifies the molecules' structural and functional characteristics to some extent. The principle underlying PEF extraction of valuable compounds is electroporation (pore formation in the membrane). Cell sap, which is used to remove cellular contents, is released as a result of it. PEF's homogeneity, which is made possible by treating both the surface and all of the cells within, is its main advantage [26].

High Intensity Pulsed Electric Field (HIPEF)

Similar to PEF, HIPEF is an extraction method that uses high-voltage pulses applied between two electrodes. The food business can use the HIPEF process, which yields a high volume in a short amount of time [27]. This method was utilized to extract ES calcium malate. 1 g of ES powder had malic acid addition (50 mL). At a rate of 25 ml/min, the mixture was fed into the HIPEF extraction system, and the pulse generator was turned on for 120 seconds. After being extracted, the product was centrifuged to separate the solid from the liquid. The synthesis of calcium malate, which enhances calcium absorption in vitro, was shown to be energy-efficient, easy to use, and cost-effective using the HIPEF technology [19]. PEF is economical, but it is challenging to operate, non-continuous, and has a small treatment capacity [29].

Electric Discharge-Assisted Mechanical Milling (EDAMM)

In a reaction tank for EDAMM equipment, ES powder is fed between two stainless steel electrodes. One electrode holds the ES in place while another vibrates it at a frequency of 10 Hz to break it up. Utilizing an alternating current and vibrations to create an electric discharge between stainless steel electrodes and the chamber walls, the ES was changed **(Figure 3)**. In this experiment, ES is ground for 15 minutes in atmospheres that include air, argon, and nitrogen. In the presence of argon, the majority of the calcite to calcium oxide conversion will occur. Full calcium oxide recovery was achieved using the EDAMM strategy in 15 minutes [24]. Fine ceramic and metallic powders, as well as a variety of chemical reactions, have all been successfully prepared via mechanical milling. The products produced by milling operations include reactive chemicals, large-surface-area catalysts, quasi-crystalline, amorphous, and non-crystalline materials, as well as some supersaturated solid solutions [23]. EDAMM produces faster responses as well as innovative synthesis and processing techniques using low current and high voltage. Calcium oxide can be fully replenished only 15 minutes after using EDAMM. Similar results were obtained using conventional calcium carbonate calcination. Calcium carbonate must be converted to calcium oxide at a high temperature of 900°C [22].

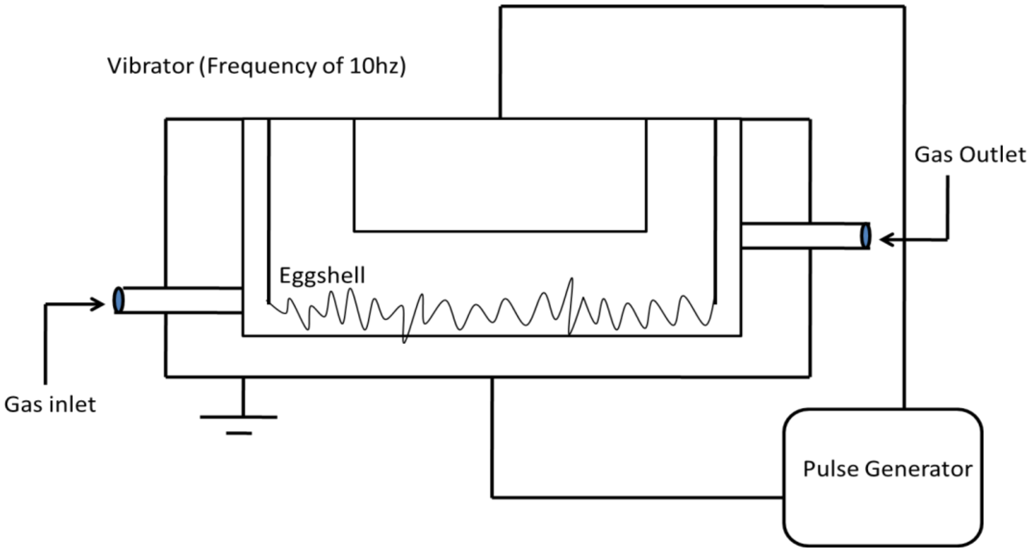

Figure 3. Electric discharge-assisted mechanical milling (EDAMM) for calcium extraction

High Energy Milling (HEM)

When using metal powders to make a variety of products in the fields of powder metallurgy and mineral processing, milling is a frequent method [14]. HEM entails dissolving large molecules into smaller ones while maintaining their clumping properties [25]. HEM is a well-known method for changing the structure and properties of materials. These modifications, which are brought on by the buildup of mechanical energy, can be identified using thermal analysis [26]. Utilizing mechanical activation and high-energy milling, ES powder is treated **(Figure 4)**. 50 tungsten carbide balls with a diameter of 10 mm and 5 g of ES are placed inside the mill [22]. It spins at 500 revolutions per minute for about 480 minutes when there is air present. After every milling cycle (ranging from 1 to 480 minutes), the machine was stopped, and 0.5 g of ES powder was removed and tagged with the milling cycle's duration.

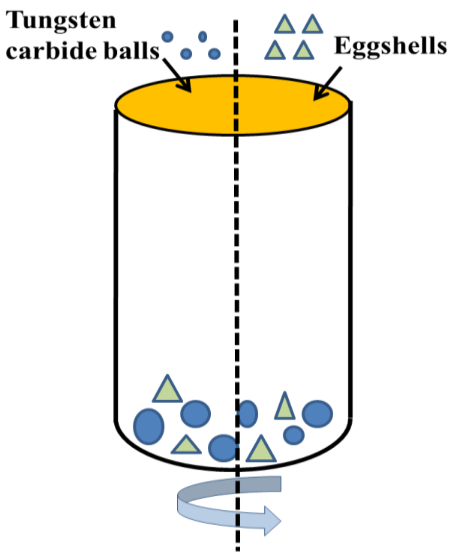

Figure 4. High energy milling for calcium extraction

Patented Products of ES Calcium

The production of novel biomaterials has begun with the use of shell and ES membranes. Many therapeutic applications have made use of the manufacture of bioactive materials based on calcium phosphate (CaP) structures employing ES as the primary calcium source [20-22]. Patented research has been summarized in **Table 1** [31-49].

Table 1. Relevant Patents for ESs

Title of Patent	Application number	References
ES calcium liquid	JPH06303949A	[31]
ES calcium composition	JPH0956363A	[32]
Manufacturing method for oxygen ES calcium powder	TW200820908A	[33]

Method of preparing calcium propionate using ES	CN1288125C	[34]
ES calcium composition and its production and food containing the ES calcium composition	JP2000093123A	[35]
Container structure of active oxygen ES calcium	TWM314638U	[36]
ES calcium composition	JPH09220071A	[37]
ES calcium oxide powder and preparation and application thereof	CN101209112B	[38]
A kind of method preparing composite organic acid calcium magnesium salts with ES, shell and magnesium oxide	CN104223100B	[39]
Calcium preparation obtained from the ES	PL238906B1	[40]
Method for manufacturing ESs calcium solubility in water using ESs	KR20150028061A	[41]
A method of liquid calcium acetate fertilizer is prepared using ES	CN109912319A	[42]
Calcium absorption enhancer	TW200916107A	[43]
Method for preparing novel organic calcium using ES	CN101024605A	[44]
Highly active calcium oxide and powdering agent	JPH0717711A	[45]
Egg white peptide chelated calcium and preparation method thereof	CN113575966A	[46]
Egg-shell ion powder and method for producing the same	JP2006246706A	[47]
ES calcium malate chewable tablets and preparation method thereof	CN102150776B	[48]
Method for fabricating calcium phosphate and calcium phosphate fabricated by using the same	WO2007126211A1	[49]

Conclusion

The ES membrane is a promising natural biomaterial that has potential uses in the fields of nutraceuticals, medicine, bioremediation, supporting chemical processes, and cosmetics. Despite its many benefits, ES membranes cannot be altered in terms of their size, shape, or thickness, and the fact that they are insoluble due to the existence of disulfide constraints in their molecular structure significantly limits the variety of applications for ES membranes. However, soluble ES membrane protein (SEP) has been suggested as an alternative biomaterial in numerous investigations. The method for making SEP relies on the dissolving of the ES membrane through the reductive cleavage of disulfide bonds, which changes the ES membrane's shape, thickness, and solubility. SEP has been widely applied in the biomedical industry and tissue engineering. Since Ovocalyxin-36 (OCX-36), a novel potential antibacterial protein associated with the ES membrane was recently identified, there is also the possibility that ES membranes may be used in the pharmaceutical industry in the future.

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