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AUTHOR

Sunardi Sunardi

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Green Technology of Processing Chicken Egg Shell Waste into Nanocalcium for Osteoporosis Prevention: A Literature Study

Sunardi

Chemical Analyst Study Program, Faculty of Engineering, Universitas Setia Budi, Surakarta

*Corresponding author: sunardi@setiabudi.ac.id

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Abstract

Egg shells have a high enough calcium content which can be used as a source of calcium. The elderly population is increasing globally and is predicted to reach 1.5 billion by 2050. The quality of life of the elderly must be considered, for example by developing functional foods for the elderly. This literature review will discuss the development of functional foods to reduce the risk of osteoporosis in the elderly. Oxidative stress is one of the factors that accelerates the occurrence of osteoporosis. Various antioxidants, including vitamin C, vitamin E, polyphenols, or lycopene, have been shown to have antioxidant activity, thereby reducing the risk of osteoporosis. In addition, the application of nanocalcium from chicken egg shells in various food products has been reported to increase calcium intake, and its use is environmentally friendly because it can contribute to reducing food waste. The application of antioxidants and nanocalcium can be a good combination, but the amount of some antioxidants must be considered so as not to interfere with calcium bioavailability. Therefore, this literature review aims to explore functional foods for the elderly to reduce the risk of osteoporosis, especially with antioxidants and nanocalcium from chicken egg shells. Eating preferences and eating patterns of the elderly are also a consideration to determine the appropriate form of functional food for the elderly. The results presented in this literature review can be the basis for the development of new food products enriched with nanocalcium from chicken egg shells for the elderly.

Keywords: *chicken egg shell, nanocalcium, osteoporosis, absorption, bioavailability*

Abstrak

Cangkang telur mempunyai kandungan kalsium yang tinggi yang dapat dimanfaatkan sebagai sumber kalsium. Populasi lansia meningkat secara global dan diprediksi mencapai 1,5 miliar pada tahun 2050. Kualitas hidup lansia harus diperhatikan, misalnya dengan mengembangkan pangan fungsional untuk lansia. Pada literatur review ini akan dibahas pengembangan pangan fungsional untuk menurunkan risiko osteoporosis pada lansia. Stres oksidatif merupakan salah satu faktor yang mempercepat terjadinya osteoporosis. Berbagai antioksidan, termasuk vitamin C, vitamin E, polifenol, atau likopen, telah dibuktikan memiliki aktivitas antioksidan, sehingga dapat mengurangi risiko osteoporosis. Selain itu, aplikasi nanokalsium dari cangkang telur ayam dalam berbagai produk makanan telah dilaporkan dapat meningkatkan asupan kalsium, dan penggunaannya ramah lingkungan karena dapat berkontribusi untuk mengurangi limbah makanan. Aplikasi antioksidan dan nanokalsium dapat menjadi kombinasi yang baik, tetapi jumlah beberapa antioksidan harus diperhatikan agar tidak mengganggu bioavailabilitas kalsium. Oleh karena itu, literatur review ini bertujuan untuk mengeksplorasi makanan fungsional bagi lansia untuk mengurangi risiko osteoporosis, terutama dengan antioksidan dan nanokalsium dari cangkang telur ayam. Preferensi makan dan pola makan lansia juga menjadi pertimbangan untuk menentukan bentuk makanan fungsional yang sesuai bagi lansia. Hasil yang disajikan dalam literatur review ini dapat menjadi dasar untuk pengembangan produk makanan baru yang diperkaya nanokalsium dari cangkang telur ayam untuk lansia.

Kata Kunci: *cangkang telur ayam, nanokalsium, osteoporosis, absorpsi, bioavailabilitas*

1. Introduction

Osteoporosis is a major health problem in the world that ranks second, behind heart disease [1], [2]. The International Osteoporosis Foundation (IOF) states that more than 30% of women worldwide are at risk of fracture due to osteoporosis, even closer to 40%. In men, the risk of fracture is 13%. Worldwide, the incidence of fractures due to osteoporosis reaches 1.7 million people [3], [4], [5]. By 2050, it is estimated that the number will continue to increase to up to 6.3 million people. Osteoporosis sufferers in Europe [6], [7], Japan [8], [9], and America reached 75 million people [10], while in China as many as 84 million people [11], [12], [13], and there are 200 million osteoporosis sufferers worldwide [14], [15].

According to the Ministry of Health, the consequences of osteoporosis in Indonesia are already at a level that must be considered, reaching 19.7% of the population. For ages less than 70 years in Indonesia, the prevalence of osteoporosis in women is 18-30%. One in 3 women and one in 5 men are affected by osteoporosis or bone fracture [16], [17].

New findings by the International Osteoporosis Foundation (IOF) indicate that 25% of women between 50 and 80 years old in Indonesia show a predisposition to osteoporosis. Women in Indonesia face a fourfold greater risk of osteoporosis compared to men. Typically, this condition primarily impacts women after menopause [16], [17], [18].

Calcium is a vital mineral with a significant function within the body. [19], [20], [21], [22]. Calcium is commonly consumed in the shape of tiny particles. The absorption of calcium by the body is associated with the size of these particles. Small calcium particles, in micro-scale, are absorbed only at a 50% rate, potentially leading to deficiencies. There's a necessity to improve technology to produce even smaller calcium particles, which would boost calcium absorption. Nano-technology presents a promising path for crafting these calcium particles. One preventive strategy for osteoporosis includes supplementing calcium [23], [24], [25]. Calcium is a crucial mineral with a significant function in the body, particularly in the formation of bones ([19], [21], [22]) Calcium is frequently ingested in the form of micro-sized particles. The body's absorption of calcium is linked to its particle size, typically resulting in only 50% absorption, which can often lead to deficiencies [26], [27], [28]. Research and development have focused on engineering smaller calcium particles to enhance their absorption in the body [29], [30], [31]. Nanoparticle synthesis has attracted more attention due to better performance caused by increased surface area and absorption power. Nano calcium has a size of 10^{-9} m [32], [33], [34]. The extremely small size ensures rapid absorption by receptors, allowing nano calcium to be absorbed by the body almost completely, close to 100% [27], [35], [36], [37], [38].

To obtain calcium in nano size, Wu et al. in 2023 have synthesized nanocalcium oxide by heating/thermal method. Calcite was heated at 900°C for 5 hours and then hydrolyzed with lime. Nanocalcium oxide obtained has a size of 50 nm [38]. Research with the microwave radiation method was carried out at a temperature of 160 °C with a time of 5 minutes to obtain nanocalcium oxide with a particle size between 14-24 nm [39]. Prayitno et al. in 2021, using the co-precipitation method for 12 hours at 40 °C and using polyvinylpyrrolidone (PVP) to prevent agglomeration obtained an average nanocalcium oxide size of 100 nm. Preparation of nanocalcium oxide using thermal decomposition method at 80 °C and argon gas resulted in particle size between 91 - 94 nm [40]. Another synthesis by chemical co-precipitation method for 60 minutes at 80°C and using polyvinyl alcohol to prevent agglomeration obtained an average particle size of 11 nm [41]. The preparation of nanocalcium oxide from shrimp shells by a two-stage process method obtained particle sizes between 40 - 130 nm [42]. Nano-sized calcium oxide particles were obtained by a 2-stage thermal decomposition method [43]. Research by Habte et al. in 2019 with the sol-gel method, namely calcination carried out at 900 °C for 1 hour, obtained nanocalcium oxide with a size of 50-198 nm.

One source of calcium is eggshells from bakery industry waste. The bakery industry uses eggs as raw materials in the process of making bread. Eggs that have been processed into bread, while the shell is thrown away. If this continues, it will have an impact on the environment. Eggshells include food waste that has not been widely utilized. The content of eggshells is 90% calcium [30], [31], [44], [45], [46], [47], [48]. This indicates that eggshells can be one of the best sources of calcium.

2. Material and Methods

Data collection from this research is secondary data by conducting a literature study on chicken eggshells that are utilized into nanocalcium for osteoporosis prevention. After obtaining the literature, a review of the literature was carried out on chicken eggshells synthesized into nanocalcium for the prevention of osteoporosis. In this study, a search for journals or scientific publications was carried out using the help of article databases, namely Google Scholar, Scencedirect, Ebsco, and Proquest. Publications used as literature study materials are limited to the last 10 years between 2014 and 2024. The keywords used were "Utilization of chicken eggshell into nanocalcium for osteoporosis prevention". The study was carried out by comparing several literatures and then arranged based on important points such as the calcium content of chicken eggshells, factors affecting the manufacture of nanocalcium from chicken eggshells, and the effects of the calcium content. So that it can be concluded that the comparison between calcium can be used as nanocalcium which can be used for the prevention of osteoporosis.

3. Results and Discussion

Synthesis of nanocalcium from chicken eggshells

One of the efforts to prevent osteoporosis is taking calcium supplements [24], [25], [53]. Calcium is a vital mineral crucial for bone formation and plays a significant role in the body [19], [20], [21], [22]). Calcium, in general, is consumed in the form of micro calcium. This size is related to the size of calcium absorption which is usually only 50% absorbed in the body, thus often causing deficiency [27], [28], [54]. The development of technology to create smaller calcium particles aims to enhance the body's absorption of calcium [29], [30], [31]. Nanoparticle synthesis has gained increased attention because of its superior performance, attributed to greater surface area and absorption capacity. Nano calcium, measuring just 10^{-9} meters, allows receptors to rapidly and efficiently enter the body. As a result, the body can absorb nearly 100% of nano calcium [27], [35].

The method of synthesizing nanocalcium oxide by thermal method (heating) has been published by Ghiasi and Malekzadeh [38]. In the study, Wu et al. synthesized nano calcium oxide by heating calcite at 900 °C with a time of 5 hours and then hydrolyzed with lime. The synthesis results obtained nanocalcium oxide measuring 50 nm in size. Another synthesis method is the microwave radiation method carried out at a temperature of 160 °C with a time of 5 minutes. This method produces nanocalcium oxide particle size of 14-24 nm [55]. Research Prayitno et al. Year 2022 which synthesized nanocalcium by co-precipitation method with a time of 12 hours at 40°C using polyvinylpyrrolidone (PVP) to prevent agglomeration. This method obtained nanocalcium oxide size of 100 nm [56]. The method of synthesizing nanocalcium oxide by thermal decomposition method (heating) temperature of 80 ° C and argon gas flow obtained particles measuring 91 - 94 nm [40]. Another nanocalcium synthesis by chemical co-precipitation method with 60 minutes at 80°C using polyvinyl alcohol so that agglomeration can be prevented. With this method, particles of 11 nm in size were obtained [41]. Preparation of nanocalcium oxide with shrimp shells using a two-stage process method produced particles measuring 40-130 nm [42], [43]. The method of synthesizing nanocalcium with sol-gel by Habte et al. is a calcination process at 900 ° C with a time of 1 hour to obtain nanocalcium oxide measuring 50 - 198 nm [46].

Some of the results of nanocalcium synthesis research as summarized in Table 1. Various methods of making nanocalcium with chicken eggshells using precipitation methods have the advantage of being simple, cheap, not requiring expensive tools, and only requires a short time and does not need additional materials. The precipitation method uses low temperatures which require less energy. Other nanocrystalline synthesis methods use higher temperatures, polymer additives, expensive equipment and take longer.

A summary of the various methods of synthesizing nanocrystals from chicken eggshells is shown in **Table 1.**

Table 1. Summary of nanocalcium synthesis from chicken eggshells

Methods	Summary	Reference
Thermal (Heating)	Synthesis of nanocalcium oxide by heating calcite at 900°C for 5 hours and then hydrolyzing it with lime. The synthesis results obtained nanocalcium oxide which is 50 nm in size.	[38]
Microwave radiation	Synthesis of nanocalcium oxide was carried out at 160 °C with a time of 5 minutes obtained nanocalcium oxide particles measuring 14 - 24 nm.	[55]
Coprecipitation	In this method, nanocalcium was synthesized by coprecipitation with 12 hours at 40°C using polyvinylpyrrolidone (PVP) to avoid agglomeration. The nanocalcium obtained is 100 nm in size	[57]
Thermal decomposition	The thermal decomposition method was carried out at 80°C and argon gas was used. The synthesized particles were 91-94 nm in size.	[40]
Chemical coprecipitation	The chemical coprecipitation method was carried out with a time of 60 minutes at 80°C and using polyvinyl alcohol to avoid agglomeration. The synthesis results obtained particles with a size of 11 nm	[41]

Methods	Summary	Reference
Two-stage process	The method of synthesizing nanocalcium oxide with shrimp shells produced particles with a size of 40 - 130 nm.	[42]
Sol-gel	In the sol-gel method, the calcination process is carried out at 900 ° C for 1 hour resulting in nanocalcium oxide measuring 50 - 198 nm.	[46]
Precipitation	The precipitation method and the combustion stage of the precipitate at 600 ° C with a time of 1 hour produced particles with a size of 10-12 nm.	[58]

Table 1 shows that with various methods chicken eggshells can be synthesized into nanocalcium which has the potential to become a source of calcium that is more easily absorbed by the body. The synthesis of nanocalcium from eggshell waste also has the potential to utilize waste that has not been maximally utilized and can be an alternative for the prevention of environmental pollution.

Prevention of osteoporosis with nanocalcium from chicken eggshells

Bone is the primary calcified tissue in vertebrates, performing various functions including mechanical support, protection, and storage [59]. Bone is composed of 10% cells, 60% mineral crystals (hydroxyapatite), and 30% organic matrix. The primary cells involved in bone remodeling are osteoblasts and osteoclasts [60]. Osteoblasts are cells that form bone and signal the development of osteoclasts. Osteoclasts, in turn, are specialized multinucleated giant cells that break down bone tissue [59]. During bone remodeling, osteoclasts break down old or damaged bone, while osteoblasts create new bone over the course of several weeks [59]. Some factors that affect bone cell activity are nutritional and cellular factors, including oxygen supply, nutrients, endocrine, cytokines, growth factors, and free radicals. If there is an imbalance between osteoblast and osteoclast activity, it will eventually lead to osteoporosis [61].

Osteoporosis is a health problem characterized by low bone mineral density, deterioration of bone microarchitecture, decreased bone mass, increased bone fragility, and is considered an age-related disorder [59]. Osteoporosis can elevate the risk of fractures and negatively impact the quality of life. Risk factors for osteoporosis are categorized into non-modifiable and modifiable factors, with oxidative stress-related factors (such as low antioxidant levels) falling under the modifiable category [60].

A significant factor contributing to osteoporosis is insufficient calcium intake, which results in suboptimal peak bone mass and poor bone mineralization [62]. Research by Cao and Rana et al. demonstrated that childhood obesity is linked to serious comorbidities, including bone loss [63], [64]. Previous studies have reported the prevalence of osteoporosis, noting that older individuals are at a higher risk, despite the availability of preventive treatments [65], [66]. Osteoporosis is more prevalent among women compared to men, with its occurrence notably increasing following menopause [62]. Postmenopausal osteoporosis, linked to estrogen deficiency, a critical hormone influencing bone mineral density, results in bone loss by elevating osteoclastic activity [67], [68], [69]. New evidence in recent years has shed light on the connection between the immune system and bone disorders like rheumatoid arthritis and osteoporosis [70] indications propose that ovariectomy triggers an immunomodulatory response, activating costimulatory T cell cytokine LIGHT (lymphotoxin-like inducible protein). This activation subsequently enhances both osteoblastogenesis and osteoclastogenesis by regulating the expression of osteoclastogenic cytokines. LIGHT has been identified as a mediator of bone loss induced by ovariectomy, indicating a potential benefit of LIGHT antagonism in individuals with postmenopausal osteoporosis.

In women aged 50 years and older, osteoporosis affects 9% in the UK, 15% in France and Germany, 16% in the USA, and 38% in Japan. Among men aged 50 years and older, it impacts 1% in the UK, 4% in Japan, 3% in Canada, and 8% in France, Germany, Italy, and Spain [71]. Elderly men with anemia and a history of fractures are at an elevated risk of osteoporosis. The majority, around 80% to 90%, of Polish patients diagnosed with osteoporosis, including those with osteoporotic fractures, do not receive appropriate pharmacological treatment [72].

The role of oxidative stress in osteoporosis

Oxidative stress is one of the risk factors for osteoporosis. An imbalance between free radical production and antioxidant capacity can cause oxidative stress, and this oxidative stress leads to the

pathogenesis of various chronic diseases [67], [69]. Free radicals are atoms or molecules with a single unpaired electron, e.g. superoxide anion (O_2^-), hydroxyl radical ($-OH$), lipoperoxide radical (LOO^-) [73]. Reactive oxygen species (ROS) or reactive nitrogen species (RNS), including peroxyxynitrite ($ONOO^-$), nitric monoxide (NO^-) and nitrogen dioxide (NO_2^-), are radicals produced in vivo [73]. Oxidative stress can cause osteoporosis through increased expression of cytokines in bone. Bone resorption by oxidative stress occurs through the activation of nuclear factor- κ B protein, which is an important mediator of tumor necrosis factor- α (TNF- α) and osteoclastogenetic activity [74].

In regular metabolic processes, Reactive Oxygen Species (ROS) are generated through the activation of different enzymes, including nicotinamide adenine dinucleotide phosphate oxidase or NADPH oxidase (a membrane enzyme), superoxide dismutase (an enzyme found in the cytoplasm), and various mitochondrial oxidases. ROS, such as superoxide and hydrogen peroxide, act as regulatory elements in osteoclastic bone resorption activity. Superoxide produced by osteoclasts contributes to bone degradation [67], [68], [69]. In certain circumstances such as vitamin D deficiency, fractures, heightened age, and increased fracture occurrences, ROS production within osteoclasts can be escalated. This elevation in ROS levels can subsequently contribute to the development of osteoporosis [68], [69], [75]. ROS has a very short half-life, making it difficult to measure. The only way to measure ROS is through the damage it causes to proteins, lipids and DNA, which is expressed as chronic diseases, including Eggshell, a prominent natural calcium source, is explored as an alternative remedy for osteoporosis. This study investigates the efficacy of zinc-activated nano powdered eggshell (Zn-NPOS) in mitigating bone loss compared to powdered oyster shell (POS) in an ovariectomized (OVX) rat model. Nanosizing notably enhances the solubility and bioavailability of oyster shell, further amplified by zinc activation. Treatment with Zn-NPOS demonstrates superior recovery from ovariectomy-induced bone loss, as evidenced by bone analysis revealing enhanced bone strength and superior trabecular architecture compared to NPOS and POS treatments. Moreover, Zn-NPOS exhibits greater efficacy in stimulating bone formation and reducing bone resorption markers. Thus, nanosizing with zinc activation presents a promising approach to enhance the effectiveness of oyster shells for osteoporosis prevention.

Utilizing eggshell offers notable nutritional advantages, particularly in its enriched calcium content. However, the efficacy of incorporating eggshell varies depending on the type of food product and its pH. When adding eggshell to bread and biscuits, considerations include the desired calcium level, acceptance of the product's sensory qualities, and importantly, the technological enhancement of dough rheology, volume, and texture. In sweet confectionery, sensory characteristics like flavor and aroma may be affected by eggshell addition. The most favorable and desired application of eggshells appears to be in dairy products such as yogurt, fried cheese, or cranberry juice. Nonetheless, limitations exist in the food industry due to potential microbiological contamination, particularly with Salmonella, and the necessity for finely grinding the shells to prevent consumer perception of their presence as "sand" in the product.

Recent studies have explored the impact of tannins on calcium bioavailability, revealing them to act as inhibitors of calcium absorption. [77]. Contrary to this, findings from Fraga-Corral et al., Tong et al., and Zhang et al. did not corroborate this claim, indicating that tannins had no discernible effect on calcium bioavailability [78], [79], [80]. According to Amalraj and Pius, an elevation in tannic acid concentration correlates with a greater reduction in the bioavailability of calcium salts. The most substantial percentage decrease was observed with calcium formate (28.1%), while the least was noted with calcium gluconate (19.6%), maintaining a 1:1 ratio between calcium salt and tannic acid. However, studies conducted by Sulaiman et al. in 2021 and Singh and Prasad in 2023, investigating the effects of calcium and iron bioavailability from selected green leafy vegetables, did not confirm that only tannic acid decreases calcium bioavailability [77].

4. Conclusion

Efforts to reduce the risk of osteoporosis in the elderly can be made through functional foods that are high in antioxidants and calcium sources. Antioxidant sources include vitamin C, vitamin E, polyphenols, and lycopene. Calcium intake is also important, and many researchers report that chicken eggshell powder has a high calcium content and can be consumed daily through its application in food products. The utilization of eggshells as a source of calcium is also beneficial to the environment. In addition, the amount of antioxidants applied in food products should also be considered so that they do not interfere with calcium absorption or can become prooxidants that have a negative impact on bone health. The addition of vitamin D, prebiotics, probiotics and synbiotics can help improve calcium absorption. Considering the food preferences and diets of the elderly worldwide, the utilization of food waste, and the reported application of chicken eggshell in various food products with their sensory analysis, it is suggested that the application

of antioxidants and calcium from chicken eggshell powder could potentially reduce the risk of osteoporosis in the elderly.

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6. Abbreviations

<i>ROS</i>	Reactive Oxygen Species
<i>RNS</i>	Reactive Nitrogen Species
<i>Zn-POS</i>	Zn-Nano Powdered Oyster Shell
<i>POS</i>	Powdered Oyster Shell
<i>NADPH</i>	Nicotinamide Adenine Dinucleotide Phosphate

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