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Review

Eggshell calcium: A cheap alternative to expensive supplements

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STRUCTURED ABSTRACT

Background: Since prehistoric times, eggs have been used as a food source by human beings. Eggs are not only a good source of nutrition, but their shells also have many nutritional and non-nutritional components. A huge amount of eggshell waste is generated globally, and these eggshells are rich source of minerals especially calcium.

Scope and approach: Calcium carbonate comprises more than 90% by weight of an eggshell. Current review highlights how to minimise eggshell waste by extracting and utilizing its calcium for food fortification and manufacturing calcium rich food sources. It also explains how calcium from eggshell can be extracted through techniques such as electric discharge assisted mechanical milling, high intensity pulsed electric field, pulsed electric field and high energy milling.

Key findings and conclusion: This review further focuses on the utilization of eggshell in food industries which ultimately would reduce the global burden of eggshell waste to some extent.

1. Introduction

According to a recent report published by World Wildlife Fund (WWF) on world food day, the pattern in which we consume, produce and waste our food is one of the greatest threats faced by planet earth. Food system alone consumes most of the natural resources and emits greenhouse gases. In order to fulfil the dietary requirements of world's population nearly 35% of land and around 70% water is used. Furthermore; to grow crops forests are being destroyed at a great pace to provide for land to cultivate crops. Regardless of all these efforts, people do not get enough food to eat particularly because much of the food is wasted. When food is wasted, all the resources are lost along with nutrients and calories present in them (TES, 2019). There are different definitions of the term food waste as provided by different organisations. *Food waste is part of food loss and refers to discarding or alternative (non-food) use of food that is safe and nutritious for human consumption along the entire food supply chain, from primary production to end household consumer level. Food waste is recognized as a distinct part of food loss because the drivers that generate it and the solutions to it are different from those of food losses* (FAO, 2014; Philippides, Sartori, Ferrari, & M'Barek, 2019). So, according to this definition the preliminary practices, which include washing, peeling, shelling, skinning, removing inedible constituents and size lessening generate food waste, which also contains some nutrients (Spiker, Hiza, Siddiqi, & Neff, 2017).

Roughly around one-third of the edible portion of the food which is considered fit for human consumption is wasted, constituting around 1.3 billion tonnes annually (FAO, 2011). One-third of all the greenhouse gases produced globally come from wasted food (TES, 2019). Food is being wasted all the way through the food supply chain, from manufacturing to the concluding phases of dispensation. In emerging and established countries much of the food is wasted. On the other hand, in third world nations, a reduced amount of food is wasted by the users. The quantity of food wasted in industrialised countries at consumer level is more than 200 million tons which is almost corresponding to the overall net food production of 230 million tons in sub Saharan Africa (FAO, 2011).

As stated beforehand, food waste goes together with nutrient loss.

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An imperative mineral that is lost is calcium which plays a pivotal role in sustaining prime bone and teeth health and other normal body functions. As an organizational constituent, calcium combines with phosphorus and institutes the mineral fraction of bone (Shang & Wu, 2019). It plays a decisive role in preventing demineralization of bones. In spite of its indispensable role in body, insufficiency of calcium is a public concern these days. Intake of calcium from dairy sources is a suitable way to satisfy the body's calcium requirements (Fayet-Moore, Cassettari, McConnell, Kim, & Petocz, 2019). There are some natural dairy and non-dairy foundations of calcium which include dairy products like milk, cheese and yoghurt. Non-dairy sources of calcium include broccoli, kale and Chinese cabbage. Apart from these some of the non-edible parts of food such as date seeds (Al-Farsi & Lee, 2008), hake bone fish (Flammini et al., 2016), silver carp fish bone powder (Hemung, Yongsawatdigul, Chin, Limphirat, & Siritapetawe, 2018) and nile tilapia (Nishimaya et al., 2016) also contain calcium, which if extracted could be of great use.

Generally, the people do not consume calcium from dietary sources in sufficient amounts as established by the clinical guidelines. Furthermore, in regions like Asia, where large number of people are suffering from lactose intolerance, it is important to devise new sources of calcium which can help in fulfilling dietary calcium requirements (Flammini et al., 2016). Supplements of calcium prescribed by the doctors are expensive, and therefore; people with low socio-economic background may find it difficult to abide by the treatment (Silva & Nabavi, 2019). Therefore, in order to increase consumption of calcium from dietary sources, calcium fortified foods are commercially available (Jia et al., 2016). These foods may be fortified with various calcium sources such as calcium obtained from milk, calcium phosphate, calcium carbonate or calcium acquired from powder of cattle bones. Among them purified calcium carbonate is widely used since it provides about 40% of calcium (Ray, Barman, Roy, & Singh, 2017) and eggshells are the largest source of calcium with an approximate calcium content of 38% (Ray et al., 2017). This calcium carbonate can be converted to different calcium containing products such as calcium phosphate and calcium citrate, each with biomedical and food industry applications (Hou et al., 2016). Tonnes of eggshells are discarded daily which ultimately end up in landfills producing methane-a powerful greenhouse gas that causes global warming (Jacobson, 2015) therefore, these can be a low-cost alternative to commercial supplements recommended by WHO (Bartter et al., 2018). They used chicken eggshells to improve nutritional status of individuals living in sub-Saharan Africa and discovered that addition of ground eggshell to traditional foods is a safe and acceptable approach to increase dietary calcium intake of the masses.

With an approach of reducing global food waste and to provide an inexpensive calcium source for fulfilment of calcium requirements the following article aims to provide a brief insight about the role of eggshell in regulating normal physiological functions of an individual, its utilization in food industry and techniques through which calcium can be extracted.

2. Eggshells

Since prehistoric times; fertilized, unfertilized and embryo eggs of chicken have been utilised by human beings as a food source and also in the treatment of variety of diseases. The fact should be kept in mind that eggshells were not primarily produced for human consumption (Murakami, Rodrigues, de Campos, & Silva, 2007), but these can be considered as capsule for life. These are enclosed with all the ingredients in precise amounts (Szeleszczuk, Kuraś, Pisklak, & Wawer, 2016) and have complete and nutritive food (Murakami et al., 2007). While these contain ions and compounds which are necessary for generation of new life and serve as the building block of new organism (Szeleszczuk et al., 2016). The main function of eggshell is to provide a physical barrier for preventing microorganisms from invading within the cell and allowing for gaseous exchange (Murakami et al., 2007). Eggshells are readily available and can be collected in large amounts (King'ori, 2011). Chicken eggshells are a waste material obtained from domestic sources such as factories which make products from eggs, hatcheries, poultry farms, restaurants and homes. Eggshells make up about 9–12% of the total egg weight. They are a good source of dietary calcium and a suitable substitute for crustacean shells.

Researchers have been looking for different ways to utilise the waste eggshells in human diet to fulfil their calcium demands; since eggshells are a good source of calcium (Ray et al., 2017). Certain clinical studies have indicated when some organisms are supplied with calcium obtained from eggshells, it results in increased bone mineral density and antirachitic effect. People suffering from osteoporosis show increased bone strengthening, reduced pain sensation and increased mobility. Eggshell calcium has exceptional bioavailability and when administered to children and adults, shows improvements in brittle nails, hair, constipation and asthma. Eggshells strengthens bone tissues, removes radioactive elements and is alsoincorporated in various supplements by pharmaceutical companies' (Wellman-Labadie, Picman, & Hincke, 2007). Furthermore; in rat studies, it was discovered that calcium obtained from eggshells powder has a high bioavailability as compared to the commercially available calcium carbonate (Świątkiewicz, Arczewska-Wlosek, Krawczyk, Puchala, & Józefiak, 2015).

2.1. Eggshells and their nutritional profile

Considering the chemical aspect of the eggshell, it is composed of water (2%) and dry matter (98%). The dry matter in turn, is composed of 93% ash and 5% crude protein (Safitri, Supriyana, & Bahiyatun, 2017). Eggshells are composed of a network of protein fibres which are associated with crystals of calcium carbonate, calcium phosphate and magnesium carbonate along with some organic substances and water (Oliveira, Benelli, & Amante, 2013).

The eggshells are also a rich source of calcium (98.2%). Magnesium and phosphorus are present in trace amounts, providing about 0.9% of each (King'ori, 2011). A 2.7 g of eggshell powder can provide about 100% of the recommended dietary intake of calcium for adults (Milbradt et al., 2015). Some other microelements like boron, copper, iron, molybdenum, sulphur, silicon and zinc are also present in eggshells (Ray et al., 2017). Apart from that 2 types of eggs are usually seen: organic eggs (brown eggshells) and inorganic eggs (white eggshells). Organic eggs are healthier as compared to inorganic eggs. A comparison of the two eggshells is indicated in Table 1.

Murakami et al. (2007) state that eggshell comprises of around 11% of the total weight of egg and constitutes around 94% of calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate and 4% other organic substances. With increasing calcium deficiency globally, there

Table 1
A comparison of the nutritional profile of 2 types of egg shells.

Constituents (%)	White egg shell powder	Brown egg shell powder	References
Moisture	0.46	0.20	(Ray et al., 2017)
Protein	3.92	5.04	
Ash	94.61	94.28	
Fat	0.35	0.08	
Calcium	34.12	33.13	
Magnesium	0.29	0.36	
Phosphorus	0.04	0.07	
Potassium	0.03	0.04	
Sodium	0.05	0.04	
Constituents (ppm)			
Copper	< 1	< 1	
Iron	22	< 1	
Manganese	< 1	< 1	
Zinc	< 1	< 1	

Table 2
World leading egg producing countries, their egg production during 2016 and their % share in global hen egg production.

Country	Hen egg production during 2016 (billion)	Share of global hen egg production during 2016 (%)	Estimated weight of eggshells (billions)	Estimated amount of calcium extracted (on basis of 38% calcium obtained from egg shell)	Reference
China	530	54.6	47.7–63.6	18.12–24.19	(FAOSTAT, 2016; Ray et al., 2017)
USA	101.95	10.5	9.18–12.23	3.45–4.65	
India	82.93	8.5	7.46–9.95	2.83–3.78	
Mexico	54.4	5.6	4.89–6.53	1.86–2.48	
Brazil	45.79	4.7	4.12–5.49	1.57–2.09	
Russia	43.09	4.4	3.88–5.17	1.47–1.96	
Japan	42.7	4.39	3.84–5.12	1.46–1.95	
Indonesia	33.21	3.4	2.99–3.99	1.36–1.52	
Iran	19.77	2	1.78–2.37	0.67–0.9	
Turkey	18.1	1.86	1.63–2.17	0.62–0.82	
Total	971.94	99.95 ≈ 100	87.47–111.2	33.24–42.26	

is increasing interest in finding new and pure sources of calcium. Calcium carbonate from flour of bones does not have the same bioavailability as that obtained from synthetic sources. Oyster shells not only provide calcium carbonate but also provide some toxic elements such as lead, aluminium, mercury and cadmium. Eggshell however does not contain any such toxic elements.

2.2. Global production of eggshells

Industries which make products from eggs produce large volumes of eggshells which in turn results in various environmental challenges (Murakami et al., 2007). The data obtained by Food and Agriculture Organisation states that China is the largest contributor to the world's hen egg production followed by the USA, India, Mexico and Brazil (FAOSTAT, 2016). Such large volumes of eggshells obtained after egg consumption are wasted and are also source of polluting our environment (King'ori, 2011). Table 2 indicates world leading egg producing countries along with their share in the egg industry and the estimated eggshell waste produced by them. Since eggshell is the richest source of calcium, the next section highlights the importance of calcium in maintaining normal physiological functions.

3. Importance of calcium in daily life

3.1. Bone health

Osteoporosis is a multifaceted condition with multifactorial origin and is characterized with asymptomatic decline in amount of bone mass per unit volume (Yedavally-Yellayi, Ho, & Patalighung, 2019). Mechanical support and structural integrity are not maintained when bone mass gets too low and as a result fractures occur even with minimal trauma (Hatta et al., 2018; Kucukler, Simsek, Turk, Arduc, & Guler, 2017; Poole et al., 2017). Ribs, humerus, pelvis, distal radius, proximal femur and distal radius are more liable to fractures. Post-menopausal white women and elderly people are more likely to develop fractures (Arnaud & Sanchez, 1990).

Bone is a metabolically active issue that is constantly remodelled or reconditioned. Bone resorption cells (osteoclasts) and bone formation cells (osteoblasts) regulate this activity. In a normal healthy adult bone, bone resorption is coupled to and balanced by bone formation and as a result there is no net change (Yaşar, Adigüzel, Arsalan, & Matthews, 2018). To increase or decrease bone mass, these two processes must become uncoupled functionally. Magnesium, phosphorus and calcium the major mineral ions of bones play a submissive role in bringing changes in bone mass. In order for normal bone mineralization and formation to occur, these mineral ions must be present in extracellular fluids at normal and appropriate concentrations (Lakhkar et al., 2013). Minerals obtained through diet help to maintain extracellular concentration of these minerals by replacing the minerals which are lost as a result of obligatory processes (excretion and egestion) or those normally distributed to bone and soft tissues (Arnaud & Sanchez, 1990). Hydroxyapatite is effective than calcium triphosphate in preventing bone loss in post-menopausal women and it has anabolic effect on bones (Hou et al., 2016). Moschonis, Katsaroli, Lyritis, and Manios (2010) suggested that 1200 mg of calcium can significantly reduce the loss of bone mineral density in arms and legs. Similarly, 1000 mg of calcium supplementation can reduce loss of bone mineral density at femoral neck, wards triangle and proximal femur in post-menopausal women (Kärkkäinen et al., 2010).

Postmenopausal osteoporosis accounts for around 80% of total osteoporosis cases in elderly females. This could be attributed to estrogen deficiency at the start of menopause which triggers synthesis of inflammatory cytokines and in turn a negative calcium balance is created in the body. To counter this problem Al Mijan, Lee, and Kwak (2014) studied the protective effect of nano-powdered eggshell on post-menopausal osteoporosis in rats and compared the results with ovariectomised rats. Rats fed with nano powdered eggshell showed 6.6% greater bone mineral density while those fed with powdered eggshell showed 2.2% greater bone density as compared to ovariectomised rats. This also illustrates that nano-powdered eggshell has higher bioavailability as compared to powdered eggshells.

3.2. Hypertension

The two frequent diseases among the aged population include hypertension and osteoporosis and both are believed to have common aetiology i.e. calcium deficiency (Ilic, Obradović, & Vujasinović-Stupar, 2013). Calcium is known to play a key role in managing hypertension and it is believed that a higher calcium intake is associated with a reduced risk of cardiovascular disease especially in postmenopausal women (Kim, Ravichandran, & Kong, 2012). Also, calcium helps in regulating blood pressure and some studies have indicated that human or laboratory animals may be protected from hypertension if they have adequate levels of calcium in their body. There was considerable reduction in pregnancy induced hypertension and pre-eclampsia when calcium intake was increased (1–2 g/day). The supplementation of diet with calcium has proven to reduce systolic and diastolic blood pressure, but people who had low calcium intake (< 0.8 g/day) were found to have a higher blood pressure. The role of calcium in reducing systolic and diastolic blood pressure requires more investigation. Hofmeyr, Lawrie, Atallah, Duley, and Torloni (2014) in a meta-analysis reported that calcium supplementation during pregnancy among women who are at risk of calcium deficiency reduces the incidence of pre-eclampsia by more than 50%. Though there is not enough data to support the use of eggshell calcium in treating calcium, but we can hope that it will yield fruitful results, since eggshell calcium has a very high bioavailability. Siemiradzka, Dolinska, and Ryszka (2018) prepared calcium supplements from eggshell and concluded that calcium citrate prepared using eggshell is readily and completely available to the body in less than 3 h.

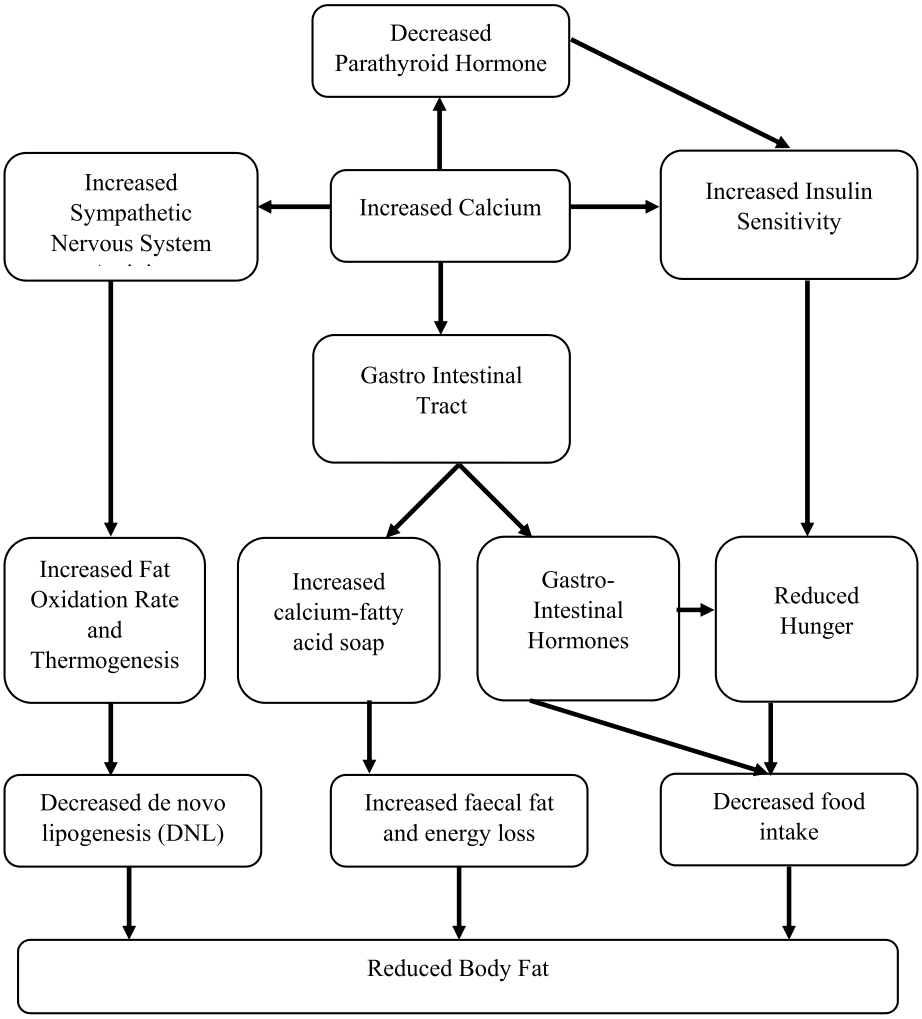

Fig. 1. A simplified scheme of anti-obesity effects of calcium and parathyroid hormone.

3.3. Weight loss

A prospective role of calcium in regulating body weight could be annotated by observations that a calcium rich diet during periods of high energy intake mitigated adipocyte lipid deposition and weight gain. A diet rich in calcium would lower down intracellular calcium ions via parathyroid hormone (Summers & Macnab, 2017). This intracellular calcium would then in return, reduce the expression of fatty acid synthase. This is a key enzyme which regulates deposition of lipid while stimulating breakdown of fat tissues. Thus; this increased oxidation of fats accounts for increased weight loss succeeding calcium. Moreover, dietary calcium increases excretion of faecal fat; thus, increasing energy losses (Ayala-Bribiesca, Turgeon, Pilon, Marette, & Britten, 2018). Fig. 1 indicates a simplified scheme of anti-obesity effect of calcium and parathyroid hormone.

Data obtained from six observational studies and controlled trials was re-evaluated to determine the effect of calcium on body weight and body fat. A consistent effect of high calcium in diet expressed itself as low body fat and low body weight and reduced weight gain in mid-life. An increment of 300 mg in dietary calcium intake was associated with around 1 kg less body fat in children and around 3 kg less body weight in adults. This was suggestive that when calcium intake was increased by 2 servings per day there was almost 70% reduction in the risks of gaining weight (Heaney, Davies, & Barger-Lux, 2002). Rats fed with nacre powder (*Pinctada maxima*) resulted in reduced fat accumulation in the body and also lowered elevated triglyceride levels (Shono et al., 2008). Hydroxyapatite obtained from sea shells and fish bones could also be incorporated as supplement (Heaney et al., 2002). Jones et al. (2013) concluded that individuals with high calcium intake had elevated levels of plasma peptide tyrosine, which in turn contributed to improved feeling of satisfaction and reduced intake of calories, hence contributing to weight loss.

Calcium obtained from eggshell has a high bioavailability as supported by the work of Dolińska, Jelińska, Szulc-Musiol, and Ryszka (2016) and Bradauskiene, Montrimaite, and Moscenkova (2017). Eggshell calcium has good solubility (Bradauskiene et al., 2017) and calcium release showed first order kinetics (rate of elimination is proportional to concentration). From tablets prepared by eggshell, around 80% of calcium (calcium citrate) was obtained within first 30 min and reached approximately 100% within 3 h. Tablets prepared with calcium carbonate showed slower release and the values obtained were 7% and 60% respectively. This area of interest requires further investigation since sufficient data is unavailable regarding the use of eggshell calcium in managing weight. But owing to different studies which support high bioavailability of eggs shell calcium, it can be postulated that eggshell calcium may replace over the counter, expensive supplements.

3.4. Colorectal cancer

In western world, colorectal cancer is the principal cause of death. Since long ago; dietary calcium has been considered beneficial in providing protection against adenomas and colorectal cancer. Calcium is

known to reduce the risk of colorectal cancer by binding to oncogenic bile acids and ionised fatty acids. This impaired the activity of these carcinogenic compounds to flourish in the mucosal layer of colon. Calcium is also known to improve the diversity or specificity of cells of colon by attaching to receptors which sense calcium. This results in a wide range of biological actions which also include the activation of isozyme protein kinase C. Modifications in expression of this protein were observed in colonic neoplastic cells in human beings and rodents. A number of clinical studies have also shown that calcium supplementation is helpful in defending the cells against pre-malignant changes in colon. There seems to be an inverse relationship between intake of calcium supplements and occurrence of distal colonic cancer (Kim et al., 2012). Bolland, Grey, Gamble, and Reid (2011) investigated post-menopausal women and suggested that there was a considerable reduction in risk of total invasive breast cancer, total breast cancer and colorectal cancer in women who consumed the prescribed amount of 1000 mg of calcium.

Though not enough work has been done by researchers to see the effectiveness of eggshell calcium powder against cancer but keeping in view the high bioavailability of eggshell calcium, and the studies supporting the efficacy of calcium against cancer, it can be hypothesized that eggshell calcium can be a suitable alternative to expensive supplements available over the counter.

3.5. Calcium signalling

In order to adapt with changing environment, cells must signal, and this signalling necessitates messengers whose concentration fluctuates with time. Calcium ions influence every facet of a cell's existence and mortality. These calcium ions join with thousands of proteins and influence changes in performance, association and localisation. Calcium is a universal dispatch rider and has a key role in nearly all types of cells including the cells which are involved in maintaining immunity such as T-lymphocytes, B-lymphocytes and mast cells. Furthermore; calcium signalling in the body is involved in controlling growth and propagation, specification and programmed cell death (apoptosis) in a wide range of programmes which encode for copying of genetic information (DNA) into a new molecule (mRNA) (Kim et al., 2012). Although, there is not sufficient data regarding the role of eggshell calcium in calcium signalling, but it is expected that calcium obtained from eggshell would counter calcium deficiency and provide calcium ions to the body for maintenance of electrolyte balance and nerve signalling. This could be concluded from the fact that calcium obtained from the eggshell has high bioavailability. Szeleszczuk, Pisklak, Kuras, and Wawer (2015) stated that dissolution of calcium carbonate obtained from eggshell was statistically significant as compared to that obtained from other sources. Their results were in line with an animal study according to which digestibility of chicken eggshell was higher and better than that of precipitated calcium carbonate (Brennan, Duncan, Wartofsky, Butler, & Wray, 1991). Table 3 illustrates some of the miscellaneous functions of calcium in the human body.

4. Extraction techniques

Eggshells are one of the most common biomaterials in nature. As a by-product of food industry, they represent a significant waste because they are discarded after utilization of egg yolk and albumin (Lesnierowski & Stangierski, 2018). Most of this waste is disposed in the landfills without any treatment beforehand. In materials science and manufacturing processes, new techniques have been developed. These techniques include electric discharge assisted mechanical milling (EDAMM) and high energy milling (HEM), pulsed electric field (PEF) and high intensity pulsed electric field (HIPEF). By using these techniques, eggshells are transformed into new materials which are suitable for utilization in advanced applications.

4.1. Pulsed electric field (PEF) and high intensity pulsed electric field (HIPEF)

PEF is a novel extraction technique that has gained tremendous interest in recent years particularly due to its cost effectiveness in pharmaceutical and food industries (Aadil et al., 2015a; Liu, Han, Zeng, Sun, & Aadil, 2016; Manzoor et al., 2019; Yogesh, 2016). Originally this technique was employed as a non-thermal technique (does not increase temperature of food during processing) and uses short pulses of electric field to inactivate micro-organisms and enzymes at room temperature with the purpose of refining the worth of food and medicinal material (Boulaaba, Kiessling, Töpfl, Heinz, & Klein, 2014; Aadil et al., 2015b, 2018). PEF can also be used to improve food safety, stabilize food commodities by inactivating their enzymes (Roobab, Aadil, Madni, & Bekhit, 2018) and to extract and recover high-value compounds from food molecules. This technique also alters functional and structural properties of the molecules to certain extent (Yogesh, 2016). The principle behind extraction of useful substances through PEF is electroporation (pore formation in membrane). It results in cell sap discharge which is used to extract cellular contents. The basic advantage of using PEF is its homogeneity; since not only the surface but also all the cells inside are pored during treatment (Siemer, Töpfl, Witt, & Ostermeier, 2018, pp. 1–12).

PEF system consists of a treatment chamber, monitoring system, fluid-handling system and a pulse generator. The treatment chamber contains electrodes and delivers high voltage to the food item. These electrodes are held in place by an insulating material which ultimately form an enclosure that contains all the food material (Ştefănoíu, Popa, Mitelut, & Popa, 2017). This technique was used to assist hydrolysis to improve extraction of calcium from fishbones (Barba et al., 2015).

Using PEF calcium citrate was extracted from eggshell by Lin, Shen, and Ye (2013). 1 g of eggshell powder was mixed with 2% citric acid

Table 3
Miscellaneous functions of calcium in body.

Role of calcium in human body	Reference
Controls nerve excitability	Robertson (2013); Pravina et al. (2013)
Helps rennin in milk coagulation in stomach	Pravina et al. (2013)
Maintains integrity of skeletal muscles	Pravina et al. (2013)
Maintains tone and contractility of heart	Robertson (2013); Pravina et al. (2013)
Decreases cellular permeability; also used in allergic conditions	Pravina et al. (2013)
Serves as a constituent of intracellular cement	Pravina et al. (2013)
Cell signalling	Zhivotovsky and Orrenius (2011)
Cell death	Zhivotovsky and Orrenius (2011)
Helps insulin to open cells to glucose	Castro et al. (2015)
Assists in fertilization	Robertson (2013)
Gene transcription	Zhivotovsky and Orrenius (2011)
Cell cycle regulation	Zhivotovsky and Orrenius (2011)
Cell proliferation	Zhivotovsky and Orrenius (2011)

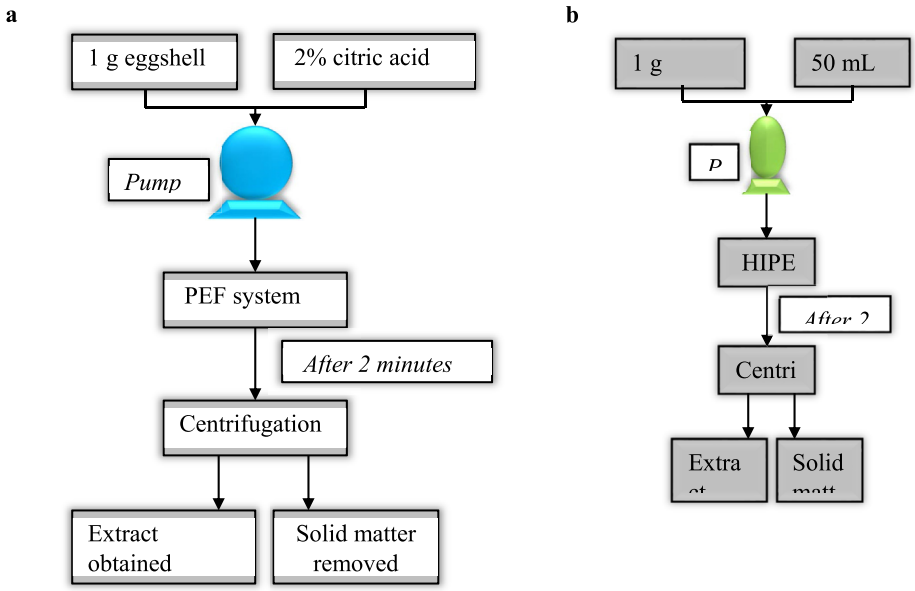

Fig. 2. a: Schematic diagram showing extraction of calcium citrate through PEF system. b: Schematic diagram showing extraction through HIPEF system.

solution and the mixture was pumped into PEF system with a flow velocity of 25 mL/min. Pulse generator was turned on and pulse duration was adjusted at 20μs with an electric field intensity of 15 kV/cm. The mixture was processed for 2 min after which voltage was turned off. The mixture was later centrifuged to obtain extraction and solid matter was removed (Fig. 2a). This calcium citrate obtained was fed to mice and the results showed that eggshell calcium citrate increased calcium absorption in mice. Moreover; this extracted calcium citrate increased bone growth and femoral deposition. Similar results were obtained by Yu, Liu, and Lin (2013) who prepared chewable tablets of eggshell calcium citrate (obtained after PEF). These tablets were then given to mice which showed increased bone calcium content and femur length.

HIPEF, like PEF is an extraction technique which involves the application of pulses of high voltage between 2 electrodes. HIPEF technique gives high yield in a short duration and this technique can be applied in food industries (He, Yin, Yan, & Yu, 2014; Wu, Zhao, Yang, & Chen, 2014; Yan, He, & Xi, 2017). This technique was used by Lin et al. (2012) to extract eggshell calcium malate. 1 g eggshell powder was mixed with malic acid (50 mL). Mixture was pumped into HIPEF extraction system at the flow velocity of 25 ml/min and pulse generator was turned on for 120 s. After taking out, the product was centrifuged and solid was removed (Fig. 2b). This solid matter was prepared to determine dissoluble calcium content of eggshell (Yan et al., 2017). Lin et al. (2012) fed calcium malate to mice which showed an increase in femoral bone deposition and bone growth as compared to mice who were not fed calcium.

Highest concentration of calcium malate was obtained when there was a combination of 20 kv/cm electric intensity, pulse duration of 24 μs and solvent concentration between 1.6 and 8.0% v/v. It was proved that HIPEF technique is cost effective, environment friendly, easy to operate and saves energy during calcium malate production which promotes calcium absorption in vitro (Yan et al., 2017). Although PEF is economically efficient; it is difficult to operate, is non-continuous and has a low treatment capacity (Boulaaba et al., 2014). While on the other hand, HIPEF has high efficiency; i.e. it provides high yield in a less time, is easy to operate, environment friendly, consumes less energy, works at a low temperature and therefore prevents loss of compounds which are sensitive to heat (He et al., 2014; Lin et al., 2012).

4.2. Electric discharge assisted mechanical milling (EDAMM)

This novel technique is used for synthesis of different high di-electric oxides (electrical insulator which can be polarized by applying an electrical field). Through EDAMM, multi-element oxides can be obtained with high purity and in a fraction of time (0.1% of the processing time required otherwise). This technique can be used to synthesize elemental oxides or multi-element oxides (Calka, Chowdhury, & Konstantinov, 2012). EDAMM ensures faster reaction by implying higher voltage electric impulses and low current. This technique is used to transform the eggshell into new material which is suitable to be used for advanced applications (Baláž, Calka, Zorkovská, & Baláž, 2013).

In a reaction vessel of EDAMM apparatus, eggshell powder is fed between two stainless steel electrodes. One electrode acts to contain the eggshell while the other vibrates at a frequency of 10 Hz to pulverize the eggshell. Alternating current was applied, and during the vibrations spaces between stainless steel electrodes and chamber walls resulted in formation of electric discharge thus, enabling transformation of eggshell (Fig. 3a). Air, argon and nitrogen are used in this experiment and eggshell is milled for 15 min in all atmospheres. Most of the transformation of calcite to calcium oxide will take place in the presence of argon. Utilization of EDAMM technique resulted in complete recovery of calcium oxide within 15 min (Baláž et al., 2013).

In order to prepare fine ceramic and metallic powders and for a wide array chemical reaction, mechanical milling turns out to be an effective technique. The types of products obtained after milling techniques include reactive chemicals, catalysts with a large surface area, quasi-crystalline, amorphous and non-crystalline materials and some supersaturated solid solutions (Calka & Wexler, 2002). EDAMM operates at low current, high voltage and provides faster reaction and new routes of synthesis and processing. Through EDAMM, 100% calcium oxide is recovered only after 15 min. These results are equivalent to ordinary calcinations of calcium carbonate. For conversion of calcium carbonate to calcium oxide, high temperature of 900 °C is required (Baláž et al., 2013). It is reported that high voltage electric discharge techniques resulted in a two-fold higher extraction yield of active ingredients (Ştefănoíu et al., 2017).

4.3. High energy milling (HEM)

Milling is a procedure which is normally used in mineral processing industries and in powder metallurgy-where wide variety of materials

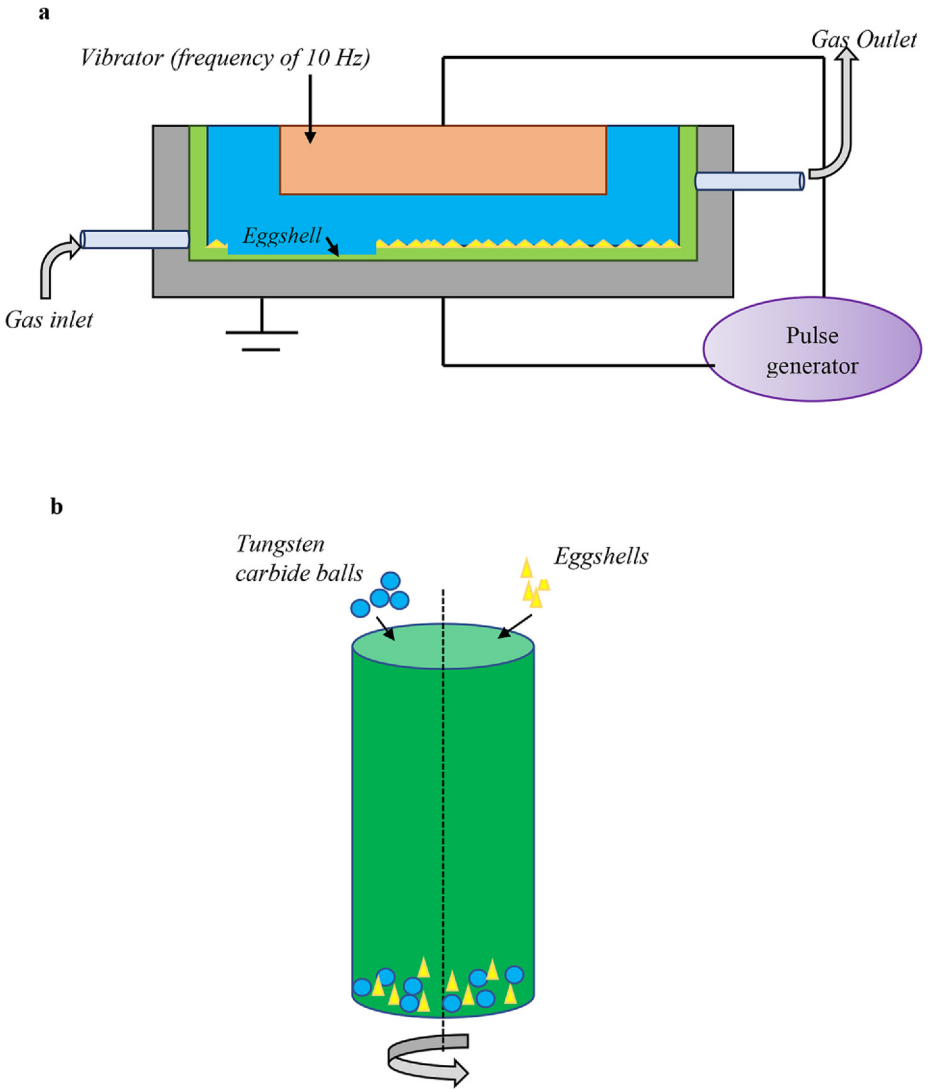

Fig. 3. a: Electric Discharge Assisted Mechanical Milling. b: High Energy Milling.

are designed from metal powders (D. L. Zhang, 2004). HEM involves mechanical breakdown of solid molecules into small molecules without affecting their state of clumping (Baláž, 2008). HEM is a known method that is used for modifying properties and structure of material. These changes are brought about by accumulation of mechanical energy and can be determined through thermal analysis (Petkova et al., 2017). Through mechanical activation by high energy milling, eggshell powder is milled (Fig. 3b). The mill is loaded with 5 g of eggshell and 50 tungsten carbide balls of 10 mm diameter (Baláž et al., 2013). At 500 revolutions per minute it is rotated for around 480 min in the presence of air. After every milling time (from 1 min to 480 min) the mill was stopped, and half gram of eggshell powder was removed and labelled according to their time of milling. After analysis it was concluded that with HEM, thermal properties and specific surface area of eggshell was considerably affected (Kostova et al., 2017; Petkova et al., 2017). Partial transformation of calcite to aragonite was obtained with excellent properties of produced material (Baláž et al., 2013).

This technique can be utilised for creation of particles of certain size and morphology and to increase surface area of solids for faster reaction (Baláž, 2008) (particularly because fine nanoparticles of nearly 40 nm are obtained (Hannora, 2014). Moreover, it increases the ratio of regions with high activity, but it is an energy intensive process and is inefficient. Almost as much as 5% of all the generated electricity is incorporated in the size reduction procedure (Baláž, 2008). Through HEM a wide variety of materials such as nano-crystalline powders, nano-composite powders, alloy powders and intermetallic powders can be made (Baláž, Zorkovská, Fabián, Girman, & Briančin, 2015; Ilieva, Dyulgerova, Petrov, Aleksandrova, & Titorenkova, 2012; Zhang, 2004). Baláž, Ficeriová, and Briančin (2016) determined the effect of milling on the adsorption capacity of eggshell and eggshell membrane. The minerals ions they used to check the adsorption capability included zinc, cadmium and silver. After milling eggshell membrane adsorbed silver ions more; while eggshells had the opposite case as they favoured adsorption of cadmium ions over silver ions.

4.4. Preparation of calcium citrate from calcium hydroxide or calcium carbonate

Eggshell which makes up about 9–12% of the weight of an egg contains around 94% calcium carbonate along with some calcium salt of phosphoric acid (calcium phosphate) and magnesium carbonate (Hassan, 2015). Calcium always exists in the from of a salt, but all the salts do not have same bioavailability. Calcium bioavailability is largely affected by stomach acid as ionisation of calcium takes place in the stomach. In less acidic environment, ionisation will not take place properly and subsequently absorption in the intestines will also be affected. Apart from low pH in stomach; magnesium, phosphorus and vitamin D levels influence intestinal absorption (Reddy, 2017). Calcium

carbonate has the highest concentration (35–40%) of elemental calcium. But despite of all these attributes, calcium carbonate shows less solubility among human beings and has low bioavailability and an inconsistent absorption rate. Since calcium carbonate is alkaline in nature, its absorption is maximum among individuals with excess stomach acid the same is the case with calcium hydroxide. Calcium citrate and calcium chloride on the other hand have an acidic nature and due to their acidic nature, they need less gastric acid for absorption (Florence, 2015). According to trials, calcium citrate showed better absorbance as compared to calcium carbonate and had a higher bioavailability (Kressel, Wolters, & Hahn, 2010).

Traditionally, there are 2 methods to prepare calcium citrate from calcium hydroxide or calcium carbonate.

- The first method involves direct reaction of calcium carbonate and citric acid
- The second method involves acid-base reaction; i.e. reaction of citric acid with calcium hydroxide (Li et al., 2016)

Different researchers have adopted different protocols to obtain calcium citrate from eggshells. For instance, Zeng and Ma (2010) extracted calcium citrate from eggshell by grinding eggshell and dissolving components with some organic acid such as acetic or lactic acid. The organic calcium thus obtained was mixed with citric acid and as a result pure calcium citrate was obtained. W. -B. Wang, Zhao, and Sun (2012) dissolved crushed eggshells with dilute hydrochloric acid until bubbles stopped forming. Filtration was carried out to separate the supernatant. Pure calcium citrate was obtained by sequentially adding sodium hydroxide and citric acid (Fig. 4).

4.5. Preparation of calcium chloride from eggshells

Calcium chloride from eggshells can be extracted by crushing eggshells and mixing with hydrochloric acid until no bubbles were seen. After centrifugation and filtration, supernatant was heated and at a temperature above 100 °C crystals of calcium chloride were obtained after drying (Garnjanagoonchorn & Changpuak, 2007). Fig. 4 shows the process for extraction of calcium chloride from eggshells. This calcium chloride, obtained from egg shell had the ability to maintain the shelf-life of fresh-cut fruits for around half a month as reported in work done by Thakur, Shaikh, Gat, and Waghmare (2019) whereas; commercially available calcium chloride increased shelf-life for up to 20 days.

5. Utilization of eggshells

5.1. Food fortification

Food fortification refers to the addition of one or more essential nutrients to the food, regardless of the fact that they are present in the food or not, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups and the primary purpose is to minimise nutritional deficiency (CAC, 2015). Calcium absorption is affected by a variety of dietary and non-dietary factors (Fig. 5). In countries where sufficient calcium is obtained from dietary sources, calcium fortification will most likely be targeted towards the at-risk population such as adolescents and post-menopausal women. Calcium status of the population can be improved by fortifying staple foods with calcium, for instance rice and rice products (Fairweather-Tait & Teucher, 2002). In addition to that snacks and beverages can also be fortified with calcium. But the bioavailability of calcium salts from various calcium fortified foods varies depending upon the product which is being fortified and the way it is consumed. Therefore; each product which is fortified must be tested individually for calcium bioavailability (Rafferty, Walters, & Heaney, 2007).

5.1.1. Factors influencing calcium absorption in the body

There are various factors which influence calcium bioavailability. These factors can be qualitative, quantitative and surrogate measures. Quantitative measures determine bioavailability of calcium from food sources through direct or indirect methods such as post prandial serum calcium determination or intestinal lavage techniques. Qualitative methods determine absorbability through supplements with the help of post prandial parathyroid hormone. Surrogate measures determine long term response of calcium bioavailability through tests like bone mineral density (Fairweather-Tait & Teucher, 2002). From nutritional aspect, bioavailability means the fraction of ingested component which is available for utilization in normal physiological functions and is determined by in vivo assays (Guerra et al., 2012). It is the result of three main steps: ability of the element to digest and solubilize in the gastrointestinal tract, absorption by intestinal cells and transportation to the circulatory system and incorporation to functional entity from circulation (Etcheverry, Grusak, & Fleige, 2012).

A protein rich diet, especially the proteins obtained from animal sources cause loss of calcium from the body. Some studies state that a diet high in animal protein results in greater bone loss. When higher amounts of sugars, proteins or fats are consumed, an insoluble complex is formed which renders calcium unavailable to the body. High sulphur to calcium ratio also increases calcium excretion from the body and hence paves the way for bone demineralization. Inadequate intake of vitamin D and high levels of dietary phosphorus and magnesium impair calcium bioavailability in the human body. Phytic acid or phytates, present in grains inhibit absorption of calcium in the body. Guéguen and Pointillart (2000) suggested that food products like bran, cereals, soya bean and seeds contain phytates and therefore reduce calcium absorption by forming an insoluble complex with calcium. Spinach, rhubarb, walnuts and sorrel contain another anti-nutritional substance called oxalates, which form an insoluble complex with calcium; thus, impairing its absorbability. Same is the case with tea, which contains tannins-a chelator which binds calcium; thus, rendering its bioavailability. Fat lipids of milk form insoluble calcium soaps; due to which they are not recommended for fortification. High salt intake also encourages calcium loss from the body. Women, who are in post-menopausal phase are more likely to develop osteoporosis because they produce less estrogen (the hormone which protects skeletal mass in young females) (Pravina, Sayaji, & Avinash, 2013).

Some physiological factors also influence calcium absorption. The parathyroid gland present in neck region is involved in maintaining body's storage of calcium. If these glands do not function properly, calcium may accumulate in the body. Likewise, in case hydrochloric acid is absent in the body, calcium will not be assimilated, since it will not be dissolved. Therefore; it won't be readily available in the body (Pravina et al., 2013). Decrease in gastric acid is reported in older age especially among women in whom a fall in oestrogen level is accompanied by a decrease in hydrochloric acid and mucin. As a result, calcium is not fully ionised and is not ionised further in the intestines because of high pH of bile salts and pancreatic juices (Reddy, 2017). This may lead to build-up of calcium in tissues and joints resulting in variety of medical complications (Pravina et al., 2013).

5.1.2. Eggshell calcium incorporation in food

Chicken eggshells can be processed using two different methods. These methods include:

- mechanical processing of dry eggshell using a mixer mill,
- converting dry eggshells into powdered by using rolling pin and sieve.

The particle size of the eggshell powder obtained by above two methods was larger than that obtained in laboratory. It was then treated with acidic solutions such as vinegar, lemon or orange juice to dissolve the eggshell. Sterilization was done prior to its incorporation in food.

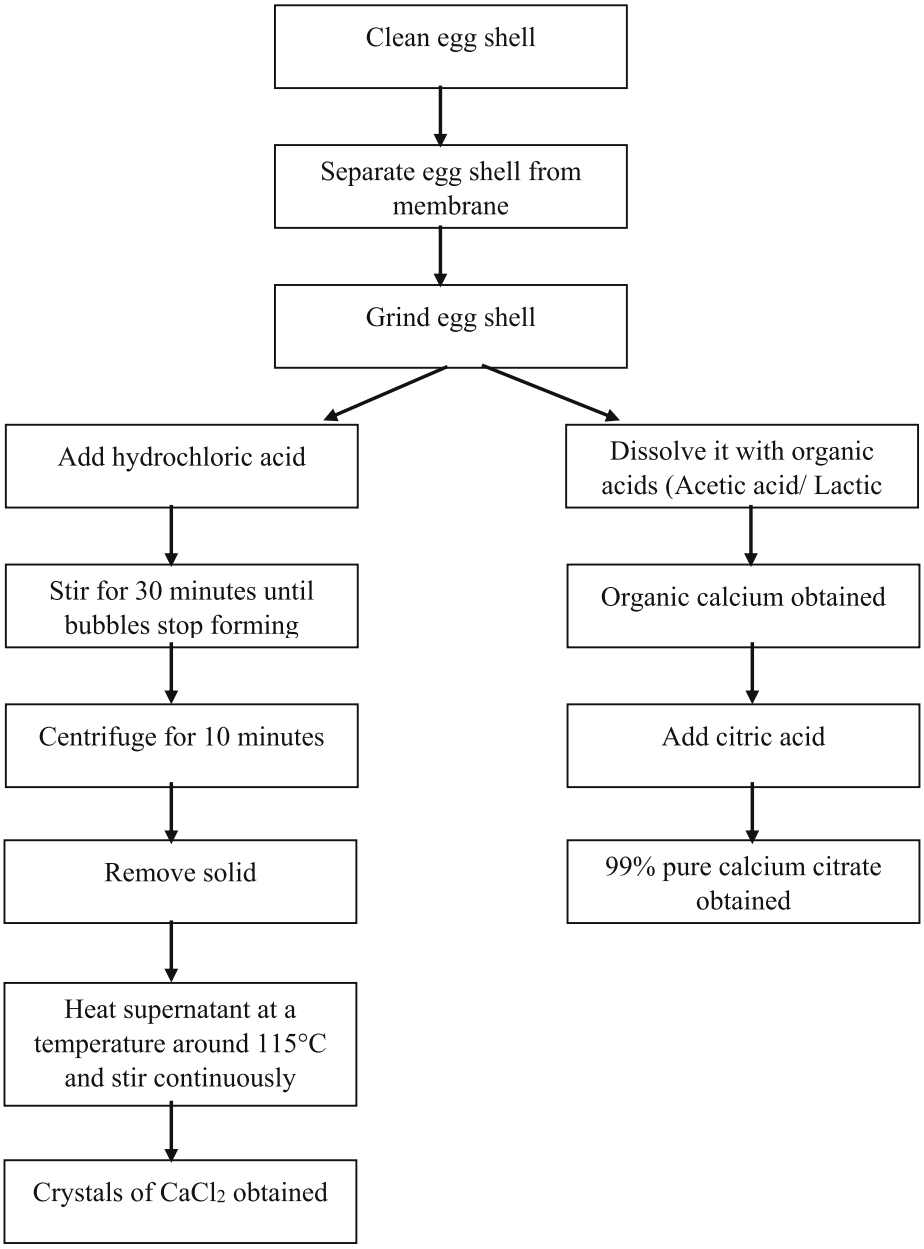

Fig. 4. Calcium citrate and $CaCl_2$ extraction.

This powder was then used to prepare calcium fortified bread, pizza, corn flour, breaded fried chicken and stew at home. Incorporation of chicken eggshell powder in the above-mentioned food products resulted in minute textural changes but no considerable effect was seen in flavour. In dishes with considerable textural changes, when particle size of eggshell was reduced, the effect was also minimised. Moreover; no considerable differences were seen in absorption of calcium obtained from eggshell powder and calcium carbonate (Brun, Lupo, Delorenzi, Di Loreto, & Rigalli, 2013). Hassan (2015) added eggshell powder to biscuits at concentrations of 3%, 6% and 9% which provided 26%, 35% and 41% of bioavailable calcium (calcium availability increased with increase in concentrations of eggshell powder). Considering texture, sensory properties and bioavailability of calcium, it was concluded that biscuits with 6% eggshell powder were best for consumption.

Ray et al. (2017) determined the effect of incorporating eggshell powder in chocolate cake at different concentrations. The eggshell powder was added at 3, 6 and 9% by weight of flour. The parameters studied included overall acceptability, mouth feel, appearance, flavour, colour and texture. The results concluded that chocolate cake, fortified with 9% eggshell powder had the highest calcium content when it was analysed chemically, and chocolate cake fortified with 3% eggshell powder showed a better overall acceptability. Sharma and Singh (2018) fortified 6 different Indian foods with calcium and vitamin D by using eggshell calcium and mushrooms respectively. The commonly used foods included chapatti, curry, daal, curd, nan-khatai, paratha and milk. Their results suggested that baked products like nan-khatai were better accepted and could be used up to 6 months.

5.2. Calcium supplementation

Some calcium supplements are available in markets which are manufactured from oyster shells such as CIPCAL-500. Extraction of calcium from oyster shells is not only tiresome but also costly since oyster shells are not readily available. So, chicken eggshells appear to be a suitable alternative source to be used in calcium supplements, as these are readily available and calcium extraction is way easier than

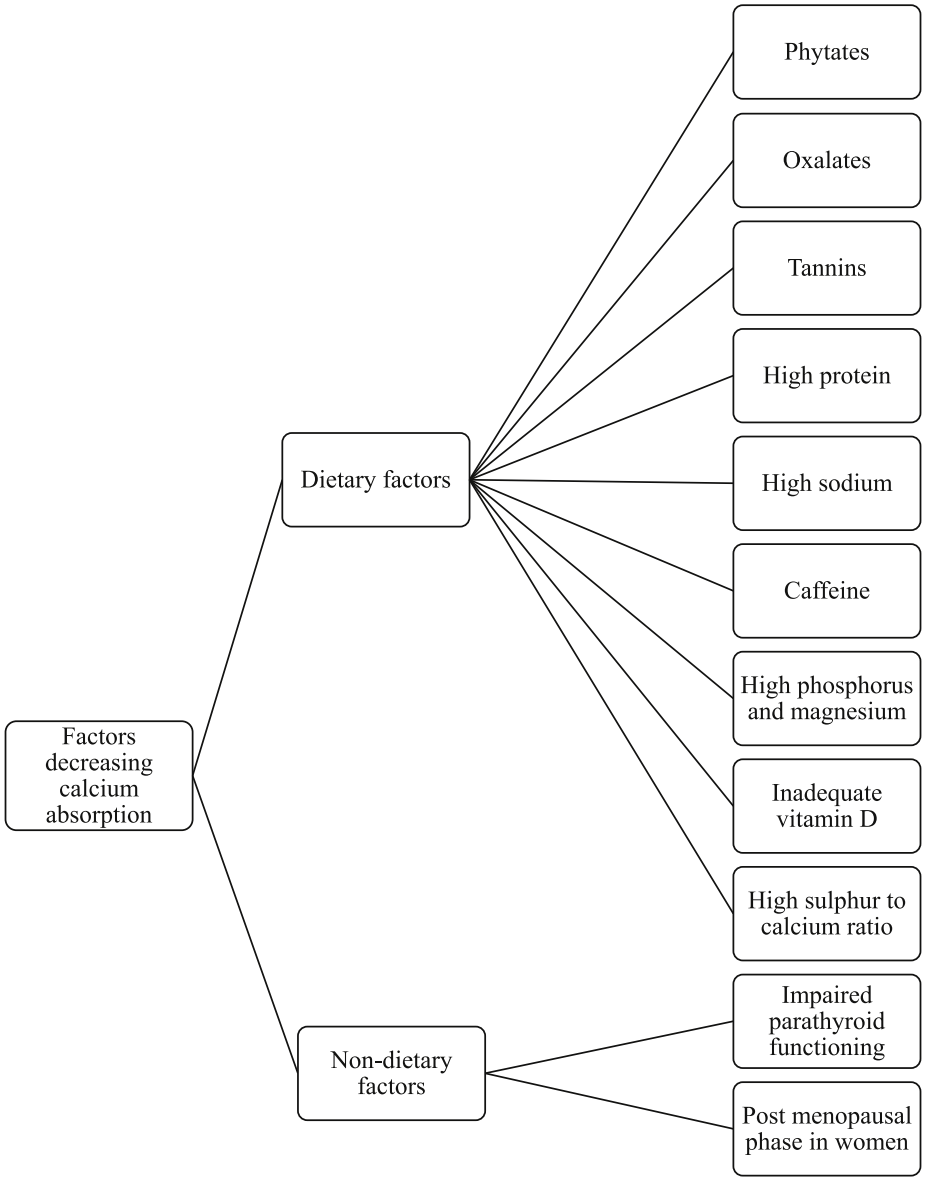

Fig. 5. Dietary and non-dietary factors influencing calcium absorption.

extraction of calcium from oyster shells. Gaonkar and Chakraborty (2016) compared extraction and bioavailability of calcium from both the sources and concluded that extraction and bioavailability of calcium obtained from chicken eggshell is easier and higher as compared to oyster shell which appears to be the main source of calcium.

5.3. Milk tablets supplemented with eggshell powder

Milk tablet is a solid product made up of milk and has several advantages. It is easy to transport and store, has low content of water, good taste, is chewable and has high bioavailability. The components of milk tablet include powdered milk, sugars (such as dextrin and sucrose) and calcium; since it is an important mineral for the human body. Calcium can be obtained from two possible sources i.e. eggshell and oyster shell. Incorporation of calcium by using nano technology results in high bioavailability of food products as compared to micro sized products. This means that the nutrients are readily available to the human body. Sensory evaluation of the milk tablets fortified with nano sized calcium powder showed that there was no difference between the control milk tablet and nano powdered eggshell fortified milk tablets (Lee, Kim, Min, & Kwak, 2016).

5.4. Preparation of lactose free milk for lactose intolerant individuals

Fina, Brun, and Rigalli (2016) prepared lactose free milk for individuals with lactose intolerance by using eggshell powder and kefir. Kefir milk is fermented milk produced by lactic acid and carbon dioxide. The milk produced does not cause lactose intolerance, prevents demineralization and is good for heart health. The researchers prepared 3 different samples and compared them with control (milk). One of the test sample contained 200 mg of eggshell powder added as a source of calcium, second sample contained milk with 2.5 g of kefir grain and third sample contained milk with the addition of 200 g eggshell powder and 2.5 g kefir grains. Results of the study illustrated that milk treated with calcium and kefir had higher calcium content and reduced lactose content, making it fit for consumption by lactose intolerant individuals.

6. Conclusion

After going through whole discussion and the available data, it can be concluded that eggshells are a valuable food stuff and have multifunctions. They are not only a source of food but also have many non-nutritive benefits. These eggshells are readily available from

households, restaurants and baking plants. More than 970 billion eggs are produced worldwide from which around 100 billion eggshells are obtained. These eggshells pose a threat to environment by causing environmental pollution. Therefore; it is important to devise ways to utilise these eggshells for nutritional and non-nutritional purposes. This would not only reduce waste but also has various economic benefits. Since eggshells are easily available they are cheap and can be an effective raw material for manufacturing various value-added food products. Calcium extraction from eggshells is easier and can be used to fortify foods with calcium to fulfil dietary calcium requirements of the population. Eggshells are also beneficial for various industries where the calcium extracted from them can be used as catalyst or as a raw material. To put it in a nut shell, eggshells have many benefits, but their full advantage is not taken. More work needs to be done in order to explore their benefits so that these can be utilised in an effective manner and their contribution in environmental pollution can be minimised.

Conflicts of interest

The authors declare no conflict of interest.

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