

## Development of Value Added Products based on Eggshell and Sun-Dried Mushrooms for the Vitamin D and Calcium Deficit Population

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### Abstract

The objective of the study was to develop a low cost calcium and vitamin D supplement from the combination of chicken eggshell and sun-dried mushrooms (*Agaricus bisporus*). Chicken eggshell can be an excellent source of calcium. Sun-dried mushrooms are a richer source of vitamin D as they contain ergosterol which gets converted to active form of vitamin D by action of UV rays of sun. The chicken eggshell and sun-dried white button mushroom powder was prepared. The calcium, vitamin D, B1, B6, C and B12 content was estimated in the developed supplement by laboratory analysis. The supplement prepared was subjected to sensory analysis to check its acceptability by incorporating it to six commonly consumed Indian food preparations items (dal curry, chapatti, parantha, curd, nankhatai and milk) as per the dosage in one serving i.e. 1 g eggshell powder: 1 g sun-dried mushrooms, keeping in mind the upper limits of intake. Results infer that the prepared supplement can be best accepted in baked food products like nankhatai etc. The shelf life of the developed supplement was found out to be for 6 months. The calcium and vitamin D supplement prepared can be promoted in the community to utilize the maximum benefits of low cost natural sources of calcium and vitamin D in comparison with traditional richer sources available in the market. Developed value added product also be used during emergencies and disasters to meet nutritional requirements of the deficit population.

**Keywords:** Calcium; Vitamin D; Chicken; Egg shell; Sun-dried mushroom

### Introduction

Calcium is the most abundant element in the human body. Calcium ions play a vital role in the physiology and biochemistry of humans and the cell as electrolytes. They play an important role in signal transduction pathways, where they act as a second messenger, in neurotransmitter release from neurons, in contraction of all muscle cell types, and in fertilization. Many enzymes require calcium ions as a cofactor. Calcium ions outside cells are also important for maintaining the potential difference across excitable cell membranes, as well as proper bone formation [1]. About 98% of the 1200 g of calcium in the adult is in the form of hydroxyapatite in the skeleton. Hydroxyapatite is a lattice-like crystal composed of calcium, phosphorus, and hydroxide. The remaining calcium is found in the extracellular fluid (50%) and in various tissues, especially skeletal muscle. Calcium is maintained within a fairly narrow range from 8.5 to 10.5 mg/dl (4.3 to 5.3 mEq/L or 2.2 to 2.7 mmol/L) [2]. There are various complications associated with calcium deficiency like:

- Metabolic bone disease
- Rickets in childhood
- Inadequate bone mass accrual in childhood and adolescence
- Inadequate foetal bone mass accrual/other metabolic effects and programming
- Possible secondary vitamin D deficiency
- Osteoporosis - postmenopausal and senile

Low dietary calcium intake further amplifies the parathyroid response to vitamin D insufficiency. The secondary hyperparathyroidism (SHPT), which ensues, mobilizes mineral and matrix from skeleton, leads to an enhanced bone loss and to a high risk of fracture and, low peak bone mass in children [3]. Active transport of calcium is dependent on the action of calcitriol and the intestinal vitamin D receptor (VDR) [4]. Normal serum vitamin D levels determine absorption of 30% of dietary calcium and more than 60-80% during periods of growth, due to the high demand for calcium. This is why vitamin D deficiency during childhood can cause delayed growth and bone abnormalities, increasing the risk of fractures in adulthood [5]. There are reports from Indian subcontinent of very low dietary intakes of calcium (<300 mg/day) causing osteomalacia, low dietary calcium and 25(OH)D status in postmenopausal women, children and pregnant women and their off springs [3].

### Chicken eggshell

In many countries around the world, egg products are being manufactured that use egg in their products generate tons of eggshells as waste product. This wastage of eggshells possess a series environmental problem due to its inadequate utilization and disposal. Eggshell weighs about 9-12% of total weight of an egg. It is made up of 94% calcium carbonate along with some magnesium carbonate and calcium phosphate. The chemical composition of chicken eggshell is 2% water and 98% dry matter. The dry matter is composed of 5% crude protein and 93% ash. Average values of mineral contents in different parts of the egg and egg shell. Eggshells contain calcium and trace amounts of other micro elements, i.e. magnesium, boron, copper, iron, manganese, molybdenum, sulphur, silicon and zinc [6]. Previous studies convey that the use of chicken eggshell powder might be

beneficial, to increase bone density and reduce pain in patients with osteoporosis. Eggshell calcium is probably the best natural source of calcium and it is about 90% absorbable [7].

## Mushrooms

Mushroom is a highly nutritional food item being rich in protein, with an important content of amino acid (e.g. leucine), fibre and low fat but with excellent important fatty acids content (e.g. linoleic acid, palmitic acid, oleic, stearic acid, arachidonic acid). Moreover, edible mushrooms provide good amount of vitamins (B1, B2, B12, C, D, and E). The most cultivated mushrooms worldwide are *Agaricus bisporus* spp. The key medicinal uses of mushrooms are antioxidant, anticancer, anti-diabetic, anti-allergic, immune-modulating, cardiovascular protector, anti-cholesterolemic, antiviral, antibacterial, anti-parasitic, anti-fungal, detoxification, and hepato-protective effects; they also protect against tumor development and inflammatory process [8].

Ergosterol occurs in milligram amounts in mushrooms, but microgram amounts of vitamin D2 have biological activity (1 µg=40 IU), so conversion of a relatively small amount of ergosterol to vitamin D2 results in a nutritionally significant increase in vitamin D [9]. A study found vitamin D2 increases in sliced white button mushrooms subjected to commercial scale UV treatment or 2.5 hr sunlight to be similar, going from 1.6 IU (0.4 µg) per 100 g in the untreated mushrooms to 1200 IU (30 µg) per 100 g for both methods of exposure [10]. Photochemical cleavage of the B ring of ergosterol takes place under UV radiation, and then the intermediate (pre-vitamin D2) formed, undergoes subsequent thermal rearrangement to form vitamin D2 (ergo-calciferol) [11].

## Materials and Methods

The study was conducted in six phases: In Phase I chicken eggshells and white button mushrooms were procured from the vendors under hygienic conditions. It was followed by Phase II which was development of chicken eggshell powder and sun-dried white button mushrooms powder. In this phase firstly chicken eggshells were processed hygienically involving following actions:

- Eggshells were washed with water gently; Firstly with tap water and then with RO water and cleaned properly with use of soft brush.
- Eggshells were boiled in water at 100 degree Celsius for 5-10 minutes.
- Eggshells were dried open air overnight.
- Eggshells were baked in oven at 180 degree Fahrenheit for 10 minutes.
- They were brought to a normal room temperature (40 degree Celsius).
- Eggshells were grinded till they became very fine in texture (powder form).

On the other side white button mushrooms were cleaned properly and then dried under sun for almost 8-10 hours with their gills upwards as a study showed a high rate of conversion of ergosterol to vitamin D2 when the mushrooms were irradiated with their gills facing the UV source [31]. They were grinder to a powdered form when they achieve cracker texture under sun.

In Phase III calcium, vitamin D and shelf life (microbiological analysis) of the developed supplement was estimated by laboratory analysis. Vitamin D was estimated using High Performance Liquid Chromatography (HPLC) and calcium content was estimated using

titration method. In the following Phase IV the developed supplement was incorporated in six popular Indian food items i.e, dal curry, chapatti, parantha, curd, nankhatai and milk. In one serving of each food item 1 g of eggshell powder and 1 g of sun-dried mushroom powder was added. The supplemented food items were sensory evaluated by 21 panelists from “Manav Rachna International Institute of Research and Studies” to check the overall acceptability of the supplement. Phase VI was statistical analysis of the data obtained using MS Excel including Mean and ANOVA-single factor. The last phase was to compare the prepared supplement with other traditional rich sources of calcium and vitamin D on the basis of calcium and vitamin D content, cost and shelf life. The comparable sources included milk, paneer, sardines and commercially available supplements in comparison with developed chicken eggshell and sun-dried mushroom powder.

## Results and Discussion

- Estimation of Vitamin D, Calcium and shelf life sun dried mushrooms and egg shell powder.

Parameters	Values
CALCIUM (mg)	912/g
VITAMIN D (mg)	0.03475/g
SHELF LIFE	6 months

**Table 1:** Estimated values of calcium and vitamin D and shelf life in the developed supplement.

Table 1 shows the estimated values of calcium, vitamin D contributed by eggshell powder and sun-dried mushroom powder. These values were estimated by various chemical testing's. Calcium content of 912 mg/g is mainly contributed by eggshell powder whereas vitamin D content of 0.03475/g is contributed by sun-dried mushrooms. However a study found that a single eggshell contains  $2.07 \pm 0.18$  g of Ca; therefore half an eggshell could provide the amount of Ca needed by adult human beings per day [12]. Therefore 1 g each of eggshell and sun-dried mushroom powder can meet maximum nutritional requirement of the population so as to prevent and cure calcium and vitamin D deficiency effectively within low cost. In relation to this a study showed that in women older than 65, there is even more benefit with vitamin D intakes of between 800 and 900 IU daily and 1200-1300 mg of calcium daily, with increased bone density, decreased bone turnover, and decreased non vertebral fractures [13].

Along with these nutrients sun-dried mushrooms also contribute some values to vitamins like B1, B6, C and B12. The values of vitamin B1, B6, C and B12 were estimated using HPLC were 0.4857, 0.05839, 0.9882 and 0.9827 mg/g. During microbiological testing it was found that total colonies of TPC, and yeast and mould were 167.50 cfu/g and 23 cfu/g respectively after 15 days. pH was recorded as 6.58 with acidity of 0.47 and moisture% by weight was 0.48%. Shelf life of 6 months ensures the long term utilization of the prepared supplement if stored in air tight container in refrigerator. Therefore this much small quantity of eggshell and sun-dried mushroom powder can provide high amount of nutritional benefits to the population.

## Sensory evaluation

Anova: Single Factor				
Summary				
Groups	Count	Sum	Average	Variance
Sample A- Dal	21	59	2.8095	2.9619
Sample B- Chapatti	21	79	3.7619	3.0904
Sample C- Parantha	21	72	3.4286	3.5571
Sample D- Curd	21	85	4.0476	2.3476
Sample E- Nankhatai	21	28	1.3333	0.7333
Sample F- Milk	21	99	4.7143	1.3143

**Table 2:** Assessment of overall acceptability of six supplemented food samples using ANOVA.

Table 2 depicts that Sample E has variance of 0.7333 that is lesser as compared to other samples therefore it shows that it is the most acceptable sample among six. Also the sum of Sample E is lesser i.e. 28 justifying its higher acceptability as maximum of subjects have extremely liked it by giving score [1]. In support to this a study suggested that the best way to use chicken eggshell as dietary calcium supplement is powdered to biscuit until 6% eggshell supplementation i.e. 1378.11 mg/100 g [6]. However in the present study Sample F has got higher sum of 99 with variance of 1.3143 therefore it represents its higher non acceptability as maximum. Subjects have disliked it extremely by giving score (Table 3) [9].

Sources	Calcium (mg/100 g)	Vitamin D (mg/ 100 g)	Shelf life	Cost (per 100 g)
Milk	121	0.00012	2 days	10.5
Paneer	476	0.00013	1 week	30
Sardines	217	0.004825	3 days	15
Supplements (average)	40000	0.56	94 weeks 2 days	530
Eggshell and sun-dried mushroom powder	91200	3.475	25 weeks 5 days	45

**Table 3:** Comparison of supplement prepared with other rich sources of calcium and vitamin D on parameters of calcium and vitamin D content, shelf life and cost.

On comparing all the richest sources on the basis of calcium and vitamin D content, shelf life and cost, it was found that eggshell and sun-dried mushroom powder is the economical with respect to its highest calcium and vitamin D content as per quantity of 100 g. Milk, paneer and sardines are cheaper but are having lowest calcium and vitamin D content and also poor shelf life. Supplements which are highly prevailing in the market are having lesser of calcium and vitamin D content as compared to the formulated supplement (Eggshell and sun-dried mushrooms powder). It was concluded by a previous study concluded that eggshell calcium was more effective in increasing bone mass than calcium carbonate in postmenopausal

Vietnamese women [7]. Vitamin D supplements available in the market only provide the vitamin D content that too lesser as compared to sun-dried mushrooms along with that white button mushroom also provide other health benefits as postulated by an animal study inferring that on intake of fruiting bodies of White Button Mushrooms there was decrease in Total Cholesterol, Low Density Lipoprotein, and Triglycerides concentrations was accompanied by a significant increase in plasma high-density lipoprotein concentrations [14]. Also supplements are costlier among all therefore every strata of Indian population cannot afford them. Therefore Eggshell and sun-dried mushroom supplement was found to be most suitable and reliable as compare to other richer sources.

## Conclusion

The developed supplement based on chicken eggshell and sun-dried white button mushrooms was highly acceptable in Nankhatai (softbread biscuits) which infers that the developed supplement can be taken with baked products maximally. The upper intake levels for calcium now range from 1000-3000 mg/d, depending on life-stage group. For vitamin D, the Upper intake levels are now 4000 IU/ d for ages 9 and older but are lower for infants and young children [15]. Therefore there is no harm in taking 1 g each of eggshell powder and sun-dried button mushroom powder daily for the deficit population. For normal population it can be taken on alternative days so as to maintain optimum serum vitamin D and Calcium Levels.

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