



Utilization of Chicken Eggshell Waste: A Potential Calcium Source for Incorporation into Vegetable Soup Mix

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Abstract: Calcium, an important mineral in bones, is widely available in the form of calcium carbonate (CaCO_3) in eggshells. However, tonnes of eggshells have been wasted every year all around the globe. The current work was aimed at valorizing the eggshell waste and incorporating it as an alternative Ca source for calcium deficient people. Soup mixes (named 1-5) were formulated by varying the ratio of starch (4-12 g) and vegetables (2-10 g) along with the constant quantity of mushroom and eggshell powder. The formulated soup mix was assessed for physiochemical properties and proximate analysis. The results showed that the soup mix 4 with 4 g vegetables, 10 g corn starch, 1 g onion, and garlic, 2 g salt and sugar, 2 g mushroom, and 1 g eggshell powder was found similar to the commercial soup mix along with 3069.095 mg/kg calcium content which meets individuals' RDA requirement. The results suggest that the formulated soup mix can act as a proper preload for all individuals and is used as a supplement for commercial calcium foods. Further research is required to increase the soup's acceptability, its bioavailability, and shelf stability.

Keywords: Eggshells, Waste Utilization, Soup Mix, Value Addition, Vitamin D

Introduction

Calcium is a vital mineral in the human body; it ultimately plays a significant role in bone formation, accounting for 99 % in the bones and teeth. Intake of calcium from dairy sources is a suitable way to satisfy the body's calcium requirements [1]. However, people do not intake a sufficient amount of calcium as per the clinical guidelines which leads to the weakening of bones in postmenopausal women and osteoporosis [2, 3]. Additionally, about 65 % of the population was reported to suffer from lactose intolerance in Asian regions, which demands new calcium supplements to fulfill dietary requirements [4]. Existing calcium tablets with crustacean shell supplements (1.25 g) are more expensive and sometimes adherence of calcium in our system to targeted treatment is less [3].

In contrast, considerable amounts of calcium sources as waste are generated from chicken eggshells (8.85 million metric tons/ annum in the year 2018). The disposal of eggshell wastes becomes the major concern all around the globe which accounts for higher landfill cost for the processing industries [5]. Additionally, the landfills also cause various impacts on the environment which must be addressed properly. According to the environmental protection agency, eggshell waste has been ranked as the 15th major pollution caused by food processing industry [6]. As, the primary component of eggshell is calcium carbonate, it can act as an excellent alternative source for dietary calcium and can be useful in increasing bone mineral density (BMD) [7,8] It has also been determined that the absorption of calcium from the eggshell is more in the artificial gastric juice compared to the synthetic calcium supplements [9].

Each egg shell contains 2.21 g of calcium carbonate which accounts for about 2.07 ± 0.18 g of calcium [3]. The ability to absorb eggshell calcium is more in patients with renal failure as it is more soluble with gastric juice because of its porosity as compared to synthetic calcium source [10]. Experts involved in an e-Delphi survey agreed that eggshells of about 500-1000 mg/day boiled for a total of 30 min would have no risk in human consumption [11]. However, the nature of addition of the eggshell into the diet is a crucial factor that must be taken into account for proper digestion and absorption of calcium. It is found that the absorption rate of fine powder eggshell (34.8 %) is reported to be greater than that of a coarse powder (21.3 %) and twice as that of synthetic calcium carbonate (13.7 %) [12]. Thus, the calcium from the fine eggshell powder can be a potential source incorporated into food materials to meet the calcium requirements. However, selection of suitable product for the incorporation of the eggshell powder is necessary to produce an idyllic food product with supplementation of calcium.

Vegetable soup mix is one of the most suitable food products as it is consumed by all age groups of individuals, including osteoporosis patients. It can fulfill social consumers' health requirements, and it also plays a vital role in human nutrition [13]. Soup mix powders can be reconstituted in water within a short period for palatability, thus retaining its nutritional properties similar to that of cooked fresh vegetables [14]. It can be stored for 6 to 12 months at room temperature [15]. Existing instant soup mixes meet the essential nutrient such as carbohydrates, proteins, dietary fibers, vitamins, minerals, essential fatty acids, and oligosaccharides [16]. However, it is devoid of the required dietary calcium responsible for the proper skeletal framework. Hence, a soup mix enhanced with calcium can serve as a nutrient supplement for calcium deficient people who suffer from lactose intolerance and osteoporosis.

As discussed earlier, the absorption of calcium by the digestive system is important in developing a calcium supplement product. In order to increase calcium absorption a vitamin D rich supplement has to be incorporated [17]. As vitamin D (calciferol) has the hormone calcitriol, it induces the synthesis of Ca binding protein (calbindin) in the intestinal cells and promotes Ca absorption [18]. The Dutch Health Council recommends 1000 mg of calcium with 800 IU/d of vitamin D for osteoporosis patients as per the International Osteoporosis Foundation [19]. The

best vegetable source of Vit D can be obtained from mushrooms exposed to Ultra violet light [20]. It has been studied that the dried button mushrooms followed by exposure to sunlight for 15-120 min contain 17.5-32.5 $\mu\text{g}/100\text{ g}$ of vitamin D₂ (ergocalciferol) [21]. The primary objective of this study is to develop a suitable calcium-enriched vegetable soup mix utilizing eggshell waste, which could meet the nutritional and sensory characteristics similar to the commercial soup mix.

Materials and Methods

Fresh eggshells (1 kg) and other fresh vegetables like carrots, green beans, onions, garlic, and button mushrooms (100 g each) were collected from a local market in Thanjavur, India. A commercial vegetable soup mix of 20 g was also bought from the supermarket and used as a control for comparison.

Formulation of Calcium-Enriched Vegetable Soup Mix

Processing of eggshell powder

The eggshell powder was prepared from the membrane-free eggshells (removed manually). The eggshells of 750 g were washed, autoclaved (134 °C, 103.42 kPa for 15 min), and dried using a hot air oven at 80 °C \pm 2 °C for 2 h [22]. Subsequently, the dried eggshells were milled at 18000 rpm for 2 min in a mixer grinder (Philips HL 2270, 750 W) to a very fine powder (average particle size of 300 μm). The eggshell powders were stored in airtight containers before the preparation of the soup mix.

Processing of other soup mix ingredients

The fresh vegetables and condiments such as carrot, beans, onion, and garlic were finely chopped and dried in a hot air oven at 70 °C for 13 h; the dried vegetables' moisture content was found to be 15.08 \pm 0.07 % dry basis (db.). The button mushrooms (100 g) were washed and sun-dried for about 24 h to obtain the RDA of 800 $\mu\text{g}/100\text{ g}$ as the button mushrooms generate vitamin D of only 10 $\mu\text{g}/100\text{ g}$ when exposed to midday sunlight for 15-120 min [23]. The dried button mushrooms were then ground to fine powder at 18000 rpm for 5 min to a particle size of 120 μm . The soups were then prepared by mixing the dried vegetables, condiments, pepper, salt, sugar, corn starch, and eggshell powder.

Formulation of the soup mix

Five different soup mixes were formulated by varying the ratio of starch (4-12 g) and vegetables (2-10 g) added to the soup mix and the constant quantity of eggshell powder and mushroom powder, as described in Table 1. The prepared soup mix was optimized by comparing it with the soup mix that are commercially available in terms of physiochemical and proximate analysis.

Table 1. Different combinations of soup mix (20 g)

Ingredients	Soup mix 1 (g)	Soup mix 2 (g)	Soup mix 3 (g)	Soup mix 4 (g)	Soup mix 5 (g)
Vegetable mix	2	4	6	8	10
Starch	12	10	8	6	4
Garlic and onion	1	1	1	1	1
Salt and sugar	2	2	2	2	2
Mushroom	2	2	2	2	2
ESP	1	1	1	1	1

Physiochemical analysis

Color

Five combinations of soup mix along with commercial soup mix, each of 20 g mixed with 150 ml of boiling water, were used for analysis. Color estimation was carried out using the Hunter Color Lab (Color Flex EZ 4510 spectrophotometer) after calibrating it with a standard white and black tile of known L, a, and b values.

Viscosity

The samples' viscosity was analyzed at three different temperatures, such as 40, 45, and 50 °C using Anton Paar Rapid Visco-Analyzer, MCR 52 based on the procedure followed by [24]. The temperatures for measurement of viscosity were selected based on the general consumable temperature range for soup. The sample was prepared by mixing different combinations of vegetable soup mix powders with definite proportions of hot water (2 g in 15 ml), i.e., 20 g in 150 ml as per the commercial soup mixes' procedure.

Moisture content

Moisture content of samples were estimated in triplicates using a halogen type moisture meter (Mettler Toledo). Each soup mix of 1.5 g was kept in the moisture meter in order to obtain the moisture on a dry basis.

Proximate analysis

The proximate composition of the prepared vegetable soup mixes was carried out by AOAC, 2002 methods and studied by comparing them with commercial soup mix. To determine the crude protein, the nitrogen percentage of samples was first obtained by the Kjeldahl method. Then the nitrogen percentage was measured followed by [25]. The fat (crude) for the samples was extracted and estimated using Pelican Socplus - SCS 06 AS with hexane as a solvent based on the method followed by [26]. The carbohydrate estimation was carried out by

the Anthrone digestion method, and concentrations were measured in the UV spectrophotometer at an optical density at 620 nm. The Ash content of the soup mixes was determined using a muffle furnace followed by [27]. The dietary fiber can be determined by boiling the moisture and fat-free samples with sulfuric acid and sodium hydroxide solution consecutively for 30 minutes and the following mixture is dried in muffle furnace at 550 °C for 2 h, cooled, and weighed again. The difference between these two weights determines the weight of the crude fiber present [27]

Energy value

The energy value was calculated using the standard formula mentioned in Eq. 1 [28].

$$\text{Energy value} \left(\frac{\text{kcal}}{\text{kg}} \right) = (\text{carbohydrates} \times 4) + (\text{fat} \times 9) + (\text{proteins} \times 4) \quad (1)$$

ICP-OES analysis

An Inductively Coupled Plasma-Optical Emission Spectrometer (Optima 3100XL made by Perkin Elmer) was used to measure calcium for the developed soup mix in powder form [29]. The measurement conditions based on the signal-to-background ratio of the least sensitive element in ICP-OES were optimized, and the spectrometer was calibrated by using the performance of calibration standards prepared (aqueous), i.e., 65 % w/v 10 mL HNO₃/100 mL, and the total analysis time was carried for 5 min, including the washing time between samples. As described, each soup mix powder of 0.5 g was homogenized and digested by adding 5 ml nitric acid (65 % w/v) and 2 ml hydrogen peroxide (30 % w/v) in a microwave [30]. The digest was transferred to a 50 mL volumetric flask after adding an internal standard of 0.1 mg/L of the standard solution. The flask was then filled with demineralized water, and then each digest was decomposed into six replicates, and two water blanks were run with each batch.

Sensory evaluation

Sensory analysis was carried out by twenty volunteers of similar age groups between 17 - 27, including equal proportions of male and female panelists. The resultant 5 soup samples were organoleptically evaluated by mixing 10 g of each soup mix with 75 ml of boiling water for sensory attributes using 5 - point hedonic scale as suggested by pepper soup [31].

Statistical analysis

All the experiments were done in triplicates. Statistical analysis was performed using SPSS statistical software (Version 20.0). One-way ANOVA established significant changes with Tukey's HSD test at a 95 % confidence level.

Results

Physiochemical analysis

Color

Color plays an essential role in the characteristic appearances. From the observation made from table 2, formulated soups 2, 3, and 5 were significantly not different ($P > 0.05$) from the commercial soup 0. A gradual rise in the lightness (L^* value) and drop in a^* and b^* value was observed from the formulated soup 1 to soup 4. However, the a^* and b^* value of the commercial soup (0) was significantly different ($p < 0.05$) from that of all the formulated soups.

Viscosity

The optimum serving temperatures of soup range is between 40 °C and 80 °C [32]. To address the influence of serving temperature on the soups' consistency, the soups' viscosity was evaluated at three targeted temperatures (40 °C, 45 °C, and 50 °C). However, there were no significant changes in the viscosity with respect to the change in temperature. In case of similarity in comparison with soup mix 0, the soup mixes 3, 4, and 5 showed no significant ($p > 0.05$); whereas, the results of soup mix 1 and 2 showed a significant fall from soup mix 0 ($p < 0.05$) when subjected to analysis.

Moisture Content

The moisture content is an important factor in the shelf-life of food products. The shelf life of foods increases with a decrease in moisture percentage; the formulated soup mixes' moisture contents range from 13.52 to 14.7 %. The moisture content (% db.) of all the five formulated soup mix powders was observed to be significantly higher than the soup mix 0 ($p > 0.05$).

Proximate Analysis

Protein

Protein, being a macronutrient for bodybuilding, can contribute to texture, color, flavor, and other properties in a formulated food [33]. The protein percentage in the formulated soup mix powders increased from 3.06 % in soup mix 5 to a maximum of 8.52 % in soup mix 1. Soup mix powders 4 (3.53%) and 5 (3.06%) were observed to be significantly not different from the commercial soup mix, 0 (3.75 %). The percentage of protein in the developed vegetable soup mix 1, 2, and 3 were significantly different (higher) from the soup mix 0 ($p < 0.05$).

Fat

The presence of fat in the diet aids in improving human health helps in energy storage, and allows humans to consume fat-soluble vitamins [34]. The percentage of fat present in the commercial soup mix was 0.59 %, and the amount of fat present in the formulated soup mixes

was in the range of 6.8 to 7.9 %. Besides, the fat contents in all the five formulated soup mixes were not significantly different from each other ($p > 0.05$).

Ash

Ash is the inorganic residue that remains after ignition or complete organic matter oxidation in the food samples [35]. It has also been studied that organic matter like vegetables dry out, and the remaining inorganic minerals remain as ash. Table 2 shows that the ash content in soup mix 5 was not significantly different from soup mix 0 ($p > 0.05$).

Crude fiber

Dietary fiber helps prevent cardiovascular diseases, constipation, and diverticulosis. Table 2 shows that the crude fiber content gradually decreased from soup mix 1 (7.25 %) to 5 (1.62 %). Based on the percentage of crude fiber present in the samples, the soup mixes 4 and 5 were found not to be significantly different from soup mix 0 ($p > 0.05$). Whereas the crude fiber in soup mix 0 is significantly lesser than soup mix 1 and 2 ($p < 0.05$).

Carbohydrate

Corn starch, being the vital ingredient in the formulation of vegetable soup mix, accounts for 91 % of carbohydrates. The carbohydrate in the formulated soup mixes was not significantly different from the commercial soup mix 0 ($p > 0.05$). However, the carbohydrates in soup mix 1 and 2 were significantly lesser than that of soup mixes 3, 4, and 5.

Energy value

The energy value for the various soup samples was calculated and listed in Table 2. No gradual differences were found in the results of the soup mix from 1 to 5. However, the commercial soup mix's energy value was observed to be higher (389.39 kcal/100g) than other formulated soup mixes.

Calcium content

Calcium content in high saline foods like the developed soup mixes can be measured using ICP OES [30]. The calcium present in soup mix 1 is 2001.98, soup mix 2 is 2127.560, soup mix 3 is 2146.055, soup mix 4 is 3069.095 mg/kg, and soup mix 5 is 3849.384 mg/kg based on the analysis using ICP-OES.

Sensory Evaluation for the Prepared Soup Samples

A sensory evaluation was conducted, and the mean value of the obtained scores was represented in Figure 1. The overall acceptability was more in commercial soup mix than other formulated soups. In contrast, the overall acceptability of soup mix 4 was observed to be next to that of the commercial soup mix. However, the overall acceptability of formulated soup mixes 4 and 5 were found to be significantly not different ($p > 0.05$) from the commercial soup mix 0 (Table 3).

Table 2. Various properties of soup mix

Various Properties		Soup mix 1	Soup mix 2	Soup mix 3	Soup mix 4	Soup mix 5	Soup mix 0
Color	L*	45.87 ± 0.37 ^b	48.2 ± 0.26 ^c	48.91 ± 0.83 ^b	51.13 ± 0.16 ^b	48.80 ± 0.37 ^a	48.85 ± 0.27 ^b
	a*	6.1 ± 0.01 ^b	5.95 ± 0.07 ^c	5.89 ± 0.03 ^{cd}	5.65 ± 0.16 ^{cd}	3.03 ± 0.07 ^d	2.08 ± 0.32 ^a
	b*	30.12 ± 0.07 ^b	28.9 ± 0.13 ^c	28.67 ± 0.23 ^c	28.16 ± 0.68 ^c	22.04 ± 0.27 ^d	20.72 ± 0.41 ^a
Viscosity Pa. s	40 °C	0.183 ± 0.08 ^a	0.237 ± 0.02 ^a	0.352 ± 0.08 ^a	0.232 ± 0.04 ^a	0.350 ± 0.06 ^a	0.347 ± 0.13 ^a
	45 °C	0.178 ± 0.01 ^{bc}	0.143 ± 0.03 ^{bc}	0.446 ± 0.09 ^c	0.326 ± 0.02 ^a	0.316 ± 0.03a ^b	0.334 ± 0.08 ^c
	50 °C	0.078 ± 0.06 ^a	0.169 ± 0.03 ^b	0.252 ± 0.01 ^a	0.476 ± 0.04 ^a	0.257 ± 0.13 ^a	0.480 ± 0.09 ^b
Moisture %		14.32 ± 0.24 ^b	14.70 ± 0.15 ^b	13.86 ± 0.29 ^{bc}	13.52 ± 0.16 ^d	13.79 ± 0.22 ^{cd}	9.32 ± 0.12 ^a
Protein %		8.53 ± 0.32 ^a	7.37 ± 0.50 ^a	5.50 ± 0.66 ^b	3.53 ± 0.22 ^c	3.06 ± 0.34 ^c	3.75 ± 0.50 ^a
Fat %		7.10 ± 2.46 ^b	7.52 ± 1.45 ^b	6.98 ± 2.50 ^b	7.07 ± 3.90 ^b	6.83 ± 3.51 ^b	0.6 ± 0.07 ^a
Ash %		5.58 ± 0.1 ^d	5.69 ± 0.21 ^c	6.25 ± 0.28 ^{bc}	6.50 ± 0.25 ^{ab}	7.18 ± 0.16 ^a	7.15 ± 0.35 ^b
Carbohydrates %		56.52 ± 0.61 ^b	57.24 ± 0.91 ^b	62.03 ± 0.88 ^b	62.18 ± 1.02 ^a	62.19 ± 0.79 ^a	60 ± 0.92 ^{ab}
Crude fiber %		7.25 ± 0.35 ^a	6.49 ± 0.18 ^{bc}	3.52 ± 0.36 ^c	2.67 ± 0.14 ^d	1.62 ± 0.37 ^d	2.30 ± 0.32 ^{ab}
Energy value (kcal/100 g)		324.1	336.12	324.34	326.47	322.20	389.37

The data in the table are given as mean ± SD; Superscript a, b, c within the same column denotes significant difference (P<0.05)

Table 3. Sensory attributes of prepared soup samples

Attributes	Soup mix 1	Soup mix 2	Soup mix 3	Soup mix 4	Soup mix 5	Soup mix 0
Appearance	3.73±0.57 ^a	3.66±0.48 ^a	3.53±0.68 ^a	4.06±0.60 ^a	3.82±0.89 ^a	4.00±0.56 ^a
Consistency	3.86±0.36 ^a	4.00±0.00 ^a	3.13±0.81 ^b	4.11±0.91 ^a	4.12±0.91 ^a	3.50±0.51 ^a _b
Smell	4.00±0.00 ^a	3.33±0.81 ^b	4.73±0.57 ^a _b	4.13±0.58 ^a	3.90±0.96 ^a _b	4.00±0.79 ^a
taste	3.60±0.76 ^a	3.73±0.44 ^a	3.80±0.41 ^a	3.93±0.60 ^a	3.72±0.57 ^a	4.00±0.56 ^a
Mouthfeel	3.40±0.76 _b	3.86±0.36 ^a _b	3.46±0.75 ^b	3.60±0.56 ^b	3.61±1.03 ^a _b	4.40±0.50 ^a
Overall	3.36±0.85 ^c	3.79±0.41 ^b _c	3.66±0.48 ^b _c	4.03±0.64 ^a _b	3.97±0.60 ^a _b	4.40±0.68 ^a

Acceptability

5- like very much; 4- like slightly; 3 - neither like or dislike; 2 - dislike slightly; 1 - dislike very much

The data in the table are given as mean ± SD; Superscript a, b, c within the same column denotes significant difference (P<0.05)

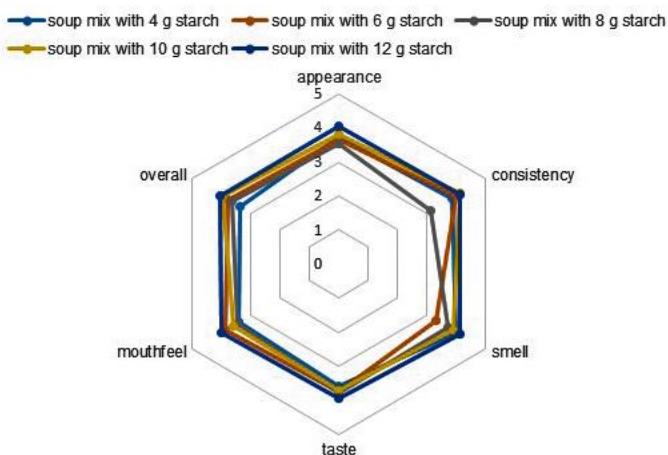


Figure 1. Sensory evaluation of formulated soup mixes

In terms of the appearance, smell, and taste, the panelists could not distinguish the soup mixes with the addition of eggshell powder. The soup mix formulations were not found to be significantly different ($p > 0.05$) from the commercial soup mix. In terms of mouthfeel, the highest score was given for the commercial soup mix 0; however, based on the scores, the formulated soup mixes 4, and 2 were observed as significantly not different ($p > 0.05$) from the commercial soup mix.

Cost Analysis

The ingredients' cost is a vital factor besides other operating factors in determining the cost of production [36]. Five samples of different soup mix ratios, each of 20 g weight, were prepared with the ingredients mentioned. Table 4 portrayed the various ingredients used in g and their cost in Dollars; it was estimated that the 100 g soup mix's preparation cost was \$1.09 (appx).

Table 4. Cost analysis chart

Ingredients	Amount in g		Cost (\$)
	Fresh	Dried	
Vegetables (carrot, beans)	100	30	0.33
Garlic and onion	50	5	0.16
Eggshell	6	5	-
Salt and sugar	10	-	0.027 (max)
Mushroom	30	10	0.30
Starch powder	40	-	0.27
Total	241	100	1.09(appx.)

Discussion

The eggshell (calcium) incorporated soup mixes with different vegetables and starch ratios were compared with the commercially available soup mix to perceive a calcium-enriched formulation, which is similar to the commercial soup mix. The soups in the liquid form were evaluated for color and viscosity; soup mixes (powder form) were evaluated for moisture, proximate analysis, and calcium.

The increase in the lightness (L^* value) from the formulated soup 1 to 4 can be correlated to the rise in the percentage of corn starch. Further, the possible reason for the gradual increase in redness (a^*) and yellowness (b^*) from the formulated soup 5 to 1 might increase the vegetable ratio from soups 5 to 1. Similar results on the influence of vegetables on the increase

in a* value of the soup was observed in a study on vegetable soup formulated with the incorporation of 0.5 % debittered moringa oleifera seed flour [25]. However, the significant difference between the a* and b* value for commercial soup compared to the formulated soups might be due to the variation in the vegetables used in the commercial soup from that of the formulated soups and also might be attributed to the addition of mushroom powder to the soup mix. The viscosity of soup was associated with the quality and quantity of starch ingredients, which acts as a thickening agent that measures its tendency to resist flow. The soups' viscosity increased with the amount of starch in the soup; when the soup mix was dispersed in water above the gelatinization temperature, it gets gelatinized and increases the soup's viscosity [37]. Thus, the presence of lower quantities of starch 4 and 6 g in the soups 1 and 2, respectively, is the reason for the decrease in the viscosity.

In case of moisture content, the average moisture present in the formulated soup mix powders were found to be above 13 % while, it has been reported in many studies that to achieve good keeping quality, the moisture contents of the dried soup mixes must be below 10 % [14, 31, 38]. Therefore, it must be essential to reduce the moisture content of vegetables (<10 %) to increase the vegetable soup powders' shelf life in the current study.

The protein content in almost all formulated soup mix powders was below 4 %; a similar range of protein content to soup mix 4 and 5 was reported by in soup mix enriched with onion, carrots, garlic, and moringa leaves [25]. The results showed that the increase in protein content from soup mix 5 to 1 might be attributed to the vegetables ratio added in the formulated soup mix. Similarly, the ash content in the formulated soup mix powders increased from soup mix 5 to 1. This could also be attributed to the percentage of vegetables and mushrooms, i.e., the higher the vegetable content, the higher the dietary fiber [39]. As reported by the percentage of crude fiber in button mushroom as 33.11 % and 38.08 % in the cape and stipe, respectively [40]. The results obtained from the crude fiber values showed that soup mix 4 and 5 were similar to that of the commercial soup mix. Further, the results (1.62 ± 0.32) of soup mix 5 intersected with the research (1.68 ± 0.01) of lentil supplemented vegetarian soup [14].

The significant difference between the fat content in the commercial soup mix and the formulated ones might be correlated to the addition of garlic and mushroom (rich in fat content) in the formulated soups. This speculation is based on the reports from a previous study conducted on mushroom soup mix, where the fat content in the dried button mushrooms was reported to be about 6.90 % [41] and on another soup mix in which the addition of garlic was accounted for the increase in the soup's total fat content. According to a study, the incorporated of onion powder, garlic powder, coriander powder, tomato powder, mint powder, cumin seed powder, dill, and Italian herb along with ridge gourd peel mix accounted for the increase in fat content which was also found to be similar to the current study [42,31].

Comparing the ash and carbohydrate content results in the formulated soup mixes, both the contents were found to increase with the increase in starch ratio added to the soup mix. For ash content, the soup mix 5 showed similar results to that of the commercial soup mix. In the case of carbohydrate, the addition of mushroom in the formulation might have also added up to the carbohydrate content to a lesser extent, as it was reported from the organic content evaluation of the mushroom soup that the mushrooms also contain a quantified amount of carbohydrate [43]. The soup mix with 40 % mushrooms showed a similar amount of carbohydrate to our formulated soup mixes [44].

The calcium present results in soup mix 4, i.e., 3069.095 mg/kg, and soup mix 5, i.e., 3849.384 mg/kg, was consistent with that of the eggshell fortified bread, having a calcium content of 3289 mg/kg [45]. A complete eggshell approximately contains 2.07 g of calcium (381mg Ca/g eggshell); therefore, half of an eggshell provides 100 % of the adult calcium daily requirement of 500-1000 mg [3]. Whereas uncooked starch also contains calcium and contributes significantly to textural properties [46]. It was observed that the uncooked starch present in the five combinations of vegetable soup mixes increases the calcium content. However, the calcium present in starch was of very trace amount, which might not satiate the human nutritional requirement. Thus, the developed vegetable soup (20 g in 150 ml water) meets the daily requirement (500-1000 mg) of humans and calcium sources from other foods. While comparing the energy values of formulated soup mixes, there was no gradual decrease or increase in the energy value. The slight fluctuations resulted from the variations in the fat, carbohydrates, and protein content due to the vegetable and starch ratios' variations. The energy value for the soup mix 4 (326.47 kcal/100 g) was similar to the results reported on the energy value (326.9 kcal/100 g) of mushroom-moringa soup [47].

Based on the sensory analysis panelists' scores, the consistency in soup mix 4 was better/thicker; this might be due to the formulation of more starch quantities compared to the vegetable chunks. The panelists found the soup mix 5 to be too starchy than other formulations, which might also be due to the ratio of starch and vegetables added in that particular soup mix. The acceptability scores of the formulated soup mix 4 were useful regardless of some minor changes like the gritty mouthfeel. The total preparation cost for 100 g soup mix was \$1.09 (appx), and the 20 g of the developed soup sample cost was estimated as \$0.21± 0.02, which was quite expensive than the commercialized product. Yet, the developed nutrient-rich soup mix fortified with calcium and vitamin D was found to be affordable.

Conclusion

Eggshell being a waste pose harmful impact on environment; however, it is rich in calcium carbonate which can be a cost-effective calcium source. Instead of polluting the environment, it can be used as an alternative to the existing commercial Ca tablets. Processing of egg shell waste and addition of vitamin D source increase calcium bioavailability. The incorporation of the processed egg shell waste into the vegetable soup mix was found to be

potential product to supplement calcium for all individuals. The developed soup mix cost was found to be affordable and could act as a useful calcium source for osteoporosis patients and lactose-intolerant people. Based on nutritional and sensory evaluation, it can be recommended that the newly formulated soup mix 4 has a sufficient amount of protein, carbohydrates, and fat as compared with the commercial soup mix. Besides, calcium and vitamin D makes the soup a suitable calcium supplement. There was a limitation i.e., gritty mouthfeel due to the presence of calcium powder in the sensory results. Further research can be done to improve the soup's palatability to make it a viable commercial product. Thus, new nutritive food formulations can be developed using low-cost resources such as the eggshell powder obtained from the poultry food chain's wastes.

References

- [1] F. Fayet-Moore, T. Cassettari, A. McConnell, J. Kim, P. Petocz, Australian children and adolescents who were drinkers of plain and flavored milk had the highest intakes of milk, total dairy, and calcium, *Nutrition Research*, 66 (2019) 68–81. <https://doi.org/10.1016/j.nutres.2019.03.001>
- [2] M. Waheed, M.S. Butt, A. Shehzad, N.M. Adzahan, M.A. Shabbir, H.A. Rasul Suleria, R.M. Aadil, Eggshell calcium: A cheap alternative to expensive supplements, *Trends in Food Science & Technology*, 91 (2019) 219–230. <https://doi.org/10.1016/j.tifs.2019.07.021>
- [3] L.R. Brun, M. Lupo, D.A. Delorenzi, V.E. Di Loreto, A. Rigalli, Chicken eggshell as suitable calcium source at home, *International Journal of Food Sciences and Nutrition*, 64 (2013) 740–743. <https://doi.org/10.3109/09637486.2013.787399>
- [4] L. Flammini, F. Martuzzi, V. Vivo, A. Ghirri, E. Salomi, E. Bignetti, E. Barocelli, Hake fish bone as a calcium source for efficient bone mineralization, *International Journal of Food Sciences and Nutrition*, 67 (2016) 265–273. <https://doi.org/10.3109/09637486.2016.1150434>
- [5] S. Owuamanam, D. Cree, Progress of Bio-Calcium Carbonate Waste Eggshell and Seashell Fillers in Polymer Composites: A Review, *Journal of composites science*, 4 (2020) 70. <https://doi.org/10.3390/jcs4020070>
- [6] M. Waheed, M. Yousaf, A. Shehzad, M. Inam-Ur-Raheem, M.K.I. Khan, M.R. Khan, N. Ahmad, Abdullah, R.M. Aadil, Channelling eggshell waste to valuable and utilizable products: A comprehensive review, *Trends in Food Science & Technology*, 106 (2020) 78–90. <https://doi.org/10.1016/j.tifs.2020.10.009>
- [7] S. Aditya, J. Stephen, M. Radhakrishnan, Utilization of eggshell waste in calcium-fortified foods and other industrial applications: A review, *Trends in Food Science & Technology*, 115 (2021) 422–432. <https://doi.org/10.1016/j.tifs.2021.06.047>

- [8] A.M. Kingori, A Review of the uses of poultry eggshells and shell membranes, *International Journal of Poultry Science*, 10 (2011) 908-912. <https://dx.doi.org/10.3923/ijps.2011.908.912>
- [9] B. Dolińska, M. Jelińska, B. Szulc-Musiół, F. Ryszka, Use of eggshells as a raw material for production of calcium preparations, *Czech Journal of Food Sciences*, 34 (2016) 313-317. <https://doi.org/10.17221/59/2016-CJFS>
- [10] D.A. Oliveira, P. Benelli, E.R. Amante, A literature review on adding value to solid residues: Egg shells, *Journal of Cleaner Production*, 46 (2013) 42-47. <https://doi.org/10.1016/j.jclepro.2012.09.045>
- [11] J. Bartter, H. Diffey, Y.H. Yeung, F. O'Leary, B. Häslar, W. Maulaga, R. Alders, Use of chicken eggshell to improve dietary calcium intake in rural sub-Saharan Africa, *Maternal Child Nutrition*, 14 (2018) 1-10. <https://doi.org/10.1111/mcn.12649>
- [12] Y. Masuda, H. Hiramatsu, (2008) Bioavailability and Physiological Function of eggshells and eggshell membranes, *John Wiley & Sons*, Tokyo. <https://doi.org/10.1002/9780470181249.ch3>
- [13] T. Farzana, S. Mohajan, T. Saha, M.N. Hossain, M.Z. Haque, Formulation and nutritional evaluation of a healthy vegetable soup powder supplemented with soy flour, mushroom, and moringa leaf, *Food Science Nutrition*, 5 (2017) 911-920. <https://doi.org/10.1002/fsn3.476>
- [14] C.P. Abeysinghe, C.K. Illeperuma, Formulation of an MSG (Monosodium Glutamate) free instant vegetable soup mix, *Journal of the National Science Foundation of Sri Lanka*, 34 (2006) 91-95. <https://doi.org/10.4038/jnsfsr.v34i2.2087>
- [15] A.M.H. Abdel-Haleem, A.A. Omran, Preparation of Dried Vegetarian Soup Supplemented with Some Legumes, *Food and Nutrition Sciences*, 5 (2014) 2274-2285. <http://dx.doi.org/10.4236/fns.2014.522241>
- [16] H. Naghedi-Baghdar, M. Nematy, M.M. Kooshyar, A. Taghipour, S.A. Sajadi Tabassi, S. Shokri, R. Javan, S.M. Nazari, Effect of a functional food (vegetable soup) on blood rheology in patients with polycythemia, *Avicenna Journal phytomedicine*, 8 (2018) 389-398.
- [17] T. Hirasawa, N. Omi, I. Ezawa, Effect of 1 α -hydroxyvitamin D₃ and egg-shell calcium on bone metabolism in ovariectomized osteoporotic model rats, *Journal of Bone and Mineral Metabolism*, 19 (2001) 84-88. <https://doi.org/10.1007/s007740170045>
- [18] H.A. Bischoff, H.B. Stähelin, W. Dick, R. Akos, M. Knecht, C. Salis, M. Nebiker, R. Theiler, M. Pfeifer, B. Begerow, R.A. Lew, M. Conzelmann, Effects of vitamin D and calcium supplementation on falls: A randomized controlled trial, *Journal Bone and Mineral Research*, 18 (2003) 343-351. <https://doi.org/10.1359/jbmr.2003.18.2.343>
- [19] A.C. Ross, J.A.E. Manson, S.A. Abrams, J.F. Aloia, P.M. Brannon, S.K. Clinton, R.A. Durazo-Arvizu, J.C. Gallagher, R.L. Gallo, G. Jones, C.S. Kovacs, S.T. Mayne, C.J. Rosen, S.A. Shapses, 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know, *Journal of*

- the Academy of Nutrition and Dietetics*, 111 (2011) 524-527. <https://doi.org/10.1016/j.jada.2011.01.004>
- [20] M.D. Kalaras, R.B. Beelman, R.J. Elias, Effects of post-harvest pulsed UV light treatment of white button mushrooms (*Agaricus Bisporus*) on vitamin D2 content and quality attributes, *Journal of Agricultural and Food Chemistry*, 60 (2012) 220-225. <https://doi.org/10.1021/jf203825e>
- [21] M.F. Holick, R.M. Biancuzzo, T.C. Chen, E.K. Klein, A.Young, D. Bibuld, R. Reitz, W. Salameh, A. Ameri, A.D. Tannenbaum, Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D, *The Journal of Clinical Endocrinology & Metabolism*, 93 (2008) 677-681. <https://doi.org/10.1210/jc.2007-2308>
- [22] H. Barakat, V. Reim, S. Rohn, Stability of saponins from chickpea, soy and faba beans in vegetarian, broccoli-based bars subjected to different cooking techniques, *Food Research International*, 76 (2015) 142-149. <https://doi.org/10.1016/j.foodres.2015.03.043>
- [23] R.R. Simon, K.M. Philips, R.L. Horst, I.C. Munro, Vitamin D mushrooms: Comparison of the composition of button mushrooms (*Agaricus bisporus*) treated post-harvest with UVB light or sunlight, *Journal of Agricultural and Food Chemistry*, 59 (2011) 8724-8732. <https://doi.org/10.1021/jf201255b>
- [24] P. Chantaro, R. Pongsawatmanit, Influence of sucrose on thermal and pasting properties of tapioca starch and xanthan gum mixtures, *Journal of Food Engineering*, 98 (2010) 44-50. <https://doi.org/10.1016/j.jfoodeng.2009.12.006>
- [25] C. Radha, B.S. Ogunsina, B.K.T. Hebina, some quality and micro-structural characteristics of soup enriched with debittered *Moringa oleifera* seeds Flour, *American Journal of Food Science and Technology*, 3 (2015) 145-149.
- [26] R.C. Kokani, S. Mokashi, Y. Pandurang Shelar, Studies on Development and Standardization of *Moringa* Leaves Instant Soup Mix Powder Incorporated with Garden Cress Seeds, *International Journal of Research and Review*, 6 (2019) 242-246.
- [27] Townshend, Official methods of analysis of the association of official analytical chemists, *Analytica Chimica Acta*, 193 (1987) 400-401. [https://doi.org/10.1016/S0003-2670\(00\)86185-1](https://doi.org/10.1016/S0003-2670(00)86185-1)
- [28] C.S. James, (1995) Experimental procedures-Estimation of major food constituents, *Analytical Chemistry of Foods*, New York, London, <https://doi.org/10.1007/978-1-4615-2165-5>
- [29] Ł. Szeleszczuk, D.M. Pisklak, M. Kuras, I. Wawer, In vitro dissolution of calcium carbonate from the chicken eggshell: A study of calcium bioavailability, *International Journal of Food Properties*, 18 (2015) 2791-2799. <https://doi.org/10.1080/10942912.2015.1004587>
- [30] Krejcova, T. Cernohorsky, D. Meixner, Elemental analysis of instant soups and seasoning mixtures by ICP - OES, *Food Chemistry*, 105 (2007) 242-247. <https://doi.org/10.1016/j.foodchem.2006.11.005>

- [31] N. Shanthini, S. Sasikala, K. Athmaselvi, Development and Evaluation of Instant Ridge Gourd Peel Soup Mix, *International Journal of Engineering and Technology*, 10 (2018) 581–585. <https://dx.doi.org/10.21817/ijet/2018/v10i2/181002102>
- [32] J.W. Kim, S.S. Samant, Y. Seo, H.S. Seo, Variation in saltiness perception of soup with respect to soup serving temperature and consumer dietary habits, *Appetite*, 84 (2015) 73–78. <https://doi.org/10.1016/j.appet.2014.09.018>
- [33] S.M. Loveday, Food Proteins: Technological, Nutritional, and Sustainability Attributes of Traditional and Emerging Proteins, *Annual Review of Food Science and Technology*, 10 (2019) 311–339. <https://doi.org/10.1146/annurev-food-032818-121128>
- [34] Michael Bockisch, (1998) The Importance of Fats, *Fats and Oils Handbook*, Academic Press and AOCS Press, 1–52. <https://doi.org/10.1016/B978-0-9818936-0-0.50006-8>
- [35] Y.P. Tang, B.L.L. Linda, L.W. Franz, Proximate analysis of *Artocarpus odoratissimus* (Tarap) in Brunei Darussalam, *International Food Research Journal*, 20 (2013) 409–415.
- [36] K. Kishor, H. Singh, S. Dey, Studies on cost of production for value added prepared mixed flour biscuit, *International Journal of Chemical Studies*, 5 (2017) 1904–1905.
- [37] Y.H. Chang, S.T. Lim, B. Yoo, Dynamic rheology of corn starch-sugar composites, *Journal of Food Engineering*, 64 (2004) 521–527. <https://doi.org/10.1016/j.jfoodeng.2003.08.017>
- [38] V. Singh, K. Kaur, Development, formulation and shelf life evaluation of baby corn soup mix from industrial by-products, *Journal of Food Science and Technology*, 57 (2020) 1917–1925. <https://doi.org/10.1007/s13197-019-04227-1>
- [39] F.Nasiri, T.B. Ghiassi, A. Bassiri, S. Hoseini, M. Aminafshar, Comparative Study on the Main Chemical Composition of Button Mushrooms (*Agaricus bisporus*) Cap and Stipe, *Journal of Food Biosciences and Technology*, 3 (2013) 41–48.
- [40] H.W. Michael, G. Bultosa, L.M. Pant, Nutritional contents of three edible oyster mushrooms grown on two substrates at Haramaya, Ethiopia, and sensory properties of boiled mushroom and mushroom sauce, *International Journal of Food Science & Technology*, 46 (2011) 732–738. <https://doi.org/10.1111/j.1365-2621.2010.02543.x>
- [41] N.L. Uju, O.P. Obiakor, Nutritional Profile of Three Different Mushroom Varieties Consumed in Amaifeke, Orlu Local Government Area, Imo State, Nigeria, *Food Science and Quality Management*, 31 (2014) 70–78.
- [42] F.S. Reis, L. Barros, A. Martins, I.C.F.R. Ferreira, Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study, *Food and Chemical Toxicology*, 50 (2012) 191–197. <https://doi.org/10.1016/j.fct.2011.10.056>
- [43] D. Chatterjee, D. Halder, A. Mukherjee, S. Das, Evaluation of Mineral and Organic Composition of Mushroom Soup, *Research Journal of Life Sciences, Bioinformatics,*

- Pharmaceutical and Chemical Sciences*, 5 (2019) 480-485.
<https://doi.org/10.26479/2019.0501.41>
- [44] A.Srivastava, B. Attri, S. Verma, (2019) Development and evaluation of instant soup premix using oyster mushroom powder, *Mushroom Research*, 28 (2019) 65-69.
<https://doi.org/10.36036/mr.28.1.2019.91960>
- [45] A.M.A. Alsuhaibani, Rheological and Nutritional Properties and Sensory Evaluation of Bread Fortified with Natural Sources of Calcium, *Journal Food Quality*, (2018) 1-7.
<https://doi.org/10.1155/2018/8308361>
- [46] A.G.M. Schulz, J.M.M. Van Amelsvoort, A.C. Beynen, Dietary Native Resistant Starch but Not Retrograded Resistant Starch Raises Magnesium and Calcium Absorption in Rats, *The Journal of Nutrition*, 123 (1993) 1724-1731.
<https://doi.org/10.1093/jn/123.10.1724>
- [47] T. Farzana, S. Mohajan, T. Saha, M.N. Hossain, Development of A Healthy Soup Powder Using Phytonutrient Enriched Mushroom-Moringa Leaf, *DIU Journal of Allied Health Sciences*, 3 (2016) 33-40.

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