



# The Technological Study to Improve Calcium Absorption of Eggshell

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## Abstract

About 110 billion tons of eggshells are produced globally and the ultimate destination is the landfills, waste utilization and these eggshells are rich source of minerals especially calcium. Calcium carbonate comprises more than 90% by weight of an eggshell [1]. The main purpose of the study to determine an appropriate condition and process for enriching absorption of calcium carbonate in chicken eggshell. In terms of that we have conducted several different kinds of studies and processes in dissolved eggshell with lemon juice and citric acid and dried it up then tested samples by technological experiments. The result revealed that:

(1) By adding dissolved eggshell to 12% of the weight of citric acid and extracting at a temperature of 4°C -6°C for 14 days and drying for 5-6 hours at 50°C is determined as the most appropriate condition.

(2) The amount of calcium contained in blood serum of the rabbit fed with test samples showed that 35.2% more than the control rabbit at 21 day, and the amount of calcium released in manure were 2.5 times less than rabbits fed with the eggshell only, indicating that calcium absorption is improving.

**Keywords:** Calcium carbonate; Lemon juice; Citric acid; Absorption

## Introduction

Calcium is an important mineral in homeostasis in all vertebrate animals. It is the most abundant mineral in the body, and is the major component of bones and teeth. In addition, calcium is involved in various vital physiologic processes, including blood coagulation, muscle contraction, membrane permeability, nerve conduction, enzyme activity, and hormone release [2].

If calcium levels in the blood drop below normal, calcium will be taken from bone and put into the blood in order to maintain blood calcium levels. Therefore, it is important to consume enough calcium to maintain adequate blood and bone calcium levels [3].

The most abundant mineral in human body is calcium, and 99% of its in teeth and bone. Consuming mineral enriched food is one of the most nutritious and essential in food consumption for human [4]. Recently, many studies have presented that food habits of Mongolian people have modified and diverse dramatically and the minerals, including calcium, which are essential for body, are quite low level in their food consumption.

- A national survey on "Assessment of Nutrition of the Population" conducted by UNICEF and the Ministry of Health in 1992 revealed that 44.7% of young children were in severe rickets condition.
- More than 10 million Americans have osteoporosis and an additional 33.6 million have osteopenia, the precursor to osteoporosis. The costs incurred from treating osteoporosis-related fractures in the United States are estimated at \$20 billion annually [5].
- Researcher of "Monhimo" Center, Researcher of Chemistry Department of the National University of Mongolia D. Demberelnyam (Ph.D) in 2014 illustrated that osteoporosis is one of the most abundant diseases in Mongolia which is in present 95% of Mongolian population.

Clinical and experimental studies show that eggshell powder has positive effects on bone and cartilage and that it is a suitable source of calcium in the prevention and treatment of osteoporosis [6]. The

clinical research on NEM began with a pilot study published in patients with mild-to-moderate joint pain received 500 mg NEM daily. After just seven days, participants had an average 26% improvement in pain and 28% improvement in flexibility. After 30 days, they had an average 73% reduction in pain, with 45% of participants reporting they were completely pain-free. They also had a 44% improvement in flexibility and 76% less pain through their range of motion. No adverse side effects were reported [7].

Japanese scientists have been produced a specific nutritious product enhancing grinded eggshell calcium by 18 amino acids, collagen and hyaluronic acid and vitamin C. Clinical studies in postmenopausal women and women with senile osteoporosis showed that eggshell powder reduces pain and osteoresorption and increases mobility and bone density or arrests its loss.

Clinical and experimental studies showed that eggshell powder has positive effects on bone and cartilage and that it is suitable in the prevention and treatment of osteoporosis [8].

Osteoporosis affects men and women of all races. But white and Asian women-especially older women who are past menopause- are at highest risk [9].

## Aim of the Study

The aim of this study is to test the samples for improving the absorption of chicken eggshell calcium. To achieve this objective, the following objectives are set. These include:

- To determine an appropriate method and condition dissolving

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calcium carbonate of eggshell in buffers

- To estimate moisture and calcium concentration in the samples
- To find out the absorption of calcium content by clinical trial

## Materials and Method

White eggshell, ascorbic acid and lemon juice from “NVTS” LLC’s “Bayan shuvuu” LLC were selected as material. The study was conducted in the biochemical accredited laboratory of the Central Laboratory, the Food Laboratory, the Laboratory of SAMO Institute, the Laboratory of Applied Science of the University of Science and Technology, the Laboratory of Microbiology and Biogeochemical Laboratories of the Mongolian University of Science and Technology, and the Zoology Laboratory of the National Science and Technology Agency.

Sample preparation: Number of eggs of Bayan shuvuu LLC, total number of eggs n=30, average weight g=57.5 ± 6 g.

Sterilizing process of eggshell was regulated by the following steps:

- To wash eggs by solution with 2% of sodium bicarbonate;
- To rinse it by flowing water;
- To keep it up to 10 minute in water at 60°C.

After that the shells were discrete from the sterilized eggs. The average weight of the shell was 7.4 ± 0.72 g. Removing the thin film from the eggshell is carefully done before boiling it in water at 93°C -96°C for 10 minute. Then drying process was performed at 93°C for 30 minute, grind about for 3 minute then filter by the 18th sieve. The boiling process for 30 min of eggshell kills any microbial growth on the surface [10].

## Results and Discussion

### Results of microbiological parameters

The result shown of bacteriological analysis of sterilized eggshell which is washed by flowing water and unwashed (Table 1).

On the table, the total number of bacterial species is greater than the permissible level both in washed and untreated samples. Citric acid greatly affects the bioavailability of Ca, Mg, P, and Zn, whereas addition of ascorbic acid enhances the bioavailability of Fe, and hesperidin boosts the bioavailability (p<0.05) of all micronutrients of the chicken eggshells [11]. We have dissolved some samples in lemon juice and citric acid, in order to improve the absorption of eggshell

calcium during the digestion process in gastro intestinal track of human. Materials for the experiments were purchased from The Bars shopping mall citric acid (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>) and lemons. The average weight of lemon is (105 ± 8 g), that can be produced around 25 ml of pure lemon juice. Citric acid and pure lemon juice contain 95% of organic acids and calcium carbonate is simply dissolvable in it therefore, it is chosen as an extraction solvent.

### Result of experiment extracting of the eggshell

The content of eggshell at 8%, 10% and 12% were dissolved and extracted in both citric acid (Experiment 2) and pure lemon juice (Experiment 1) for 14 days at 4°C-6°C (Table 2).

During the process, carbon dioxide emanated from stable gas bubbles (Figure 1).

**Experiment 1:** Pantothenic acid binds with lemon juice then produce Calcium Pantothenate, carbon dioxide and water. In 100 ml of lemon juice contains 0.2 mg of pantothenic acid. (C<sub>9</sub>H<sub>17</sub>NO<sub>5</sub>).



During the reaction carbon dioxide is produced which is identified in process of sample preparation and extraction. When eggshell is started dispersing in citric acid and pure lemon juice which induces that bubbles are been produced over the extraction much more (Figure 1).

Calcium pantothenate: Salt of pantothenic acid (vitamin B5). Commonly found in plant and animal tissue. Sometimes referred to as pantothenic acid. Biologically, pantothenic acid (and its salts) is needed to form coenzyme-A, and plays a vital role in the metabolism and synthesis of carbohydrates, proteins and fats. Additionally, pantothenic acid induces to generate new cells and tissues by effecting in the metabolism of carbohydrates and fatty acids. Furthermore, when in the body the acid is converted to pantetheine, which is crucial for a healthy pregnancy and believed to help reduce cholesterol.

**Experiment 2:** Tricarboxylic acid and calcium carbonate are involved in chemical reaction for generating carbon dioxide, calcium citrate and water.



Calcium citrate is a white, odorless powder which is sparingly soluble in water. Its molecular mass Ca<sub>3</sub>(C<sub>6</sub>H<sub>4</sub>O<sub>7</sub>)<sub>2</sub> is 498,46 g/mol.

According to the US Institute of Cancer Studies, E 333 supplement (calcium citrate) may have a potential effect to prevent deepening

No.	Specification	Acceptable amount		Unwashed (untreated)	Washed	Sterilized
		MNS ISO 4833:1995	MNS ISO 6579:1999			
1	<i>E.coli</i>	Not to be detected		Some genes of the salmonella genome was detected by PCR	Some genes of the salmonella genome was detected by PCR	undetected
2	<i>S.aureus</i>	1x10 <sup>1</sup>		undetected	undetected	undetected
3	<i>Salmonella</i> spp.	Not to be detected		detected	detected	undetected
4	Total amount of bacterial spp	1x10 <sup>4</sup>		9x10 <sup>5</sup>	4x10 <sup>5</sup>	6x10 <sup>1</sup>

Table 1: The results of bacteriological analysis of the eggshell.

No	Composition of solution	Experiment 1			Experiment 2		
		0.08	0.1	0.12	0.08	0.1	0.12
1	Eggshell, gm	8	10	12	8	10	12
2	Pure lemon juice, ml	92	90	88	-	-	-
3	Citric acid, ml	-	-	-	92	90	88

Table 2: The amount of lemon juice and acid.

cancer in the colonies and other organs of humans. Calcium citrate is the calcium salt of citric acid. It is commonly used as a food additive -E333 [12]. Calcium citrate plays also a vital role in clinical trial to release heavy metals from the body. This supplement is validated and verified to use in Europe, Russia and Ukraine. Calcium citrate is widely used in the production of jam, milk and dairy products as a stabilator of the food industry, to stabilize and inhibit from acidity and infection.

Calcium carbonate is harder to digest than calcium citrate, and calcium carbonate carries a risk of "acid rebound" (the stomach overcompensates by producing more acid), so individuals who are sensitive to antacids or who have difficulty producing adequate stomach acid may choose calcium citrate over calcium carbonate for supplementation [13].

After 14 days later, both extracts were excreted liquids on top and solids (calcium carbonate) on the bottom of beakers (Figure 2). The moisture content of the extracts evaporated and formed into solids at 50 °C for 5-5.5 h) and the results are shown in Table 3.

The highest moisture content in samples with 8% lemon juice is 86.7% and 80.4% of citric acid. As a result, the moisture content of the sample is 6.3% higher when amount of eggshell powder content is less. Samples with lemon juice were consisted moisture 1%-6% higher than samples with citric acid, so the drying time was also 30-40 minutes longer than samples with citric acid (Figure 3).

Dried preparations of solid extract with lemon juice are yellowish and the others are lighter and whiter than that.

### Results of ash and calcium concentration in dried preparations

The ash content is determined by ISO 936:2003 and the result is

shown in Figure 4.

The eggshell that was obtained as a control specimen contains 93% of calcium, and the total ash content was 96.64%.

In terms of the amount of eggshell, the ash concentration is 8% higher in citric acid sample 59.5% than the sample with lemon juice, 51.35% and the difference is 8.15%. In other samples it was decreased.

It illustrates that only the sample with 8% of citric acid is shown the closest result 70, 95% compare to calcium control sample or 0,18% less than the control sample (Table 4). In the future, we will be conducting clinical trial I to study the absorption of calcium in volunteers.

### Results of experiments in rabbits

The level of calcium in the prepared solution is determined by the MSA-500 W device (Table 5).

According to the study results, calcium levels in experiment 2 were greater than those in experiment 1, particularly in calcium samples of the version III (hereafter referred to as experimental samples) were 69.98% or 0.15-1.14 mg higher than other samples. However, the calcium contained in the III version is 1.6% less than the control, but bioactive quality of the citrate calcium is 2.5 times higher than calcium carbonate, we have chosen the optimal version. For further study on absorption of calcium content in human bodies, this sample was used on animal experiments.

We took 7 rabbits from animal testing laboratory of National Public Health Institute (1 for the control, 3 rabbits for the experiment of eggshells only and 3 rabbits for the test sample). The weight of rabbits used in the study was 1.8-2.7 kg, and fed them daily by 1.0 gr of test sample and eggshells depending on the age of the rabbit. They



A. Extraction with lemon juice



B. Extraction with citric acid

Figure 1: The extraction process.



A. Extract with lemon juice



B. Extract with citric acid

Figure 2: The extraction after 14 days.

Specification	Experiments	Content of lemon juice or citric acid	Content of moisture
Extract	Experiment 1	0.08	0.867
Solid (powder) extract			0.085
Extract		0.1	0.797
Solid extract			0.08
Extract		0.12	0.792
Solid extract			0.08
Extract	Experiment 2	0.08	0.804
Solid extract			0.085
Extract		0.1	0.791
Solid extract			0.08
Extract		0.12	0.789
Solid extract			0.08

Table 3: Moisture content of extract.



Figure 3: Dried preparations.

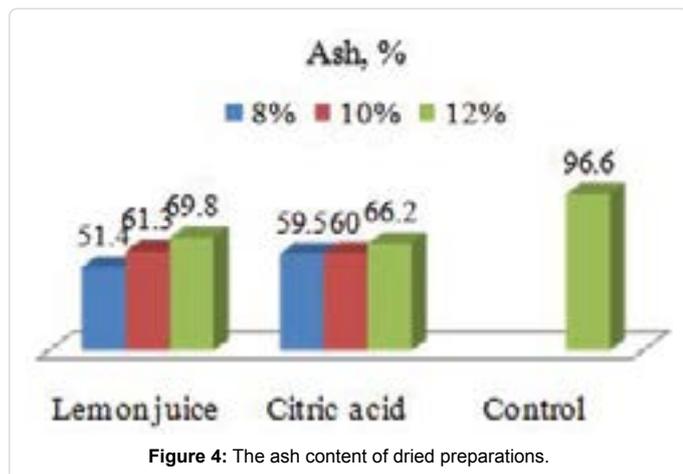


Figure 4: The ash content of dried preparations.

Control samples	Experiment 1			Experiment 2		
Eggshell	0.08	0.1	0.12	0.08	0.1	0.12
71,13	68,84	69,12	69,76	70,95	69,83	69,98

Table 4: The amount of calcium, mr%.

Version	Grinded eggshell, %	Control samples (calcined film)	Calcium solution	
			Experiment 1	Experiment 2
I	8	71.13	68.84	69.81
II	10		69.12	69.83
III	12		69.76	69.98

Table 5: The amount of calcium, mr %.

were housed individually in stainless steel cages in a standard animal facility, where the room temperature was maintained at 21°C –24°C with 40%-60% relative humidity and a 12 h light-dark cycle. The light cycle coincided with daylight hours [14].

The control rabbit was fed by the cabbage, carrots and bran [15] and remaining six rabbits were fed with eggshells only and test samples for 21 days, and the amount of calcium were determined by taking samples from blood serum and manure after 14 and 26 days (Figures 5 and 6).

The amount of calcium contained in blood serum of the rabbit fed only with eggshells was 14% lower at the 14th day than the control rabbit and 4.5% lower at the 21 day, respectively. On the other hand, rabbit fed with the test sample was 18% higher at the 14th day and 35.2% higher at 21 day of the experiment.

The parathyroid and tireocalcitonina hormones regulate the amount of calcium in the rabbit's blood. Eggshells only meals, calcium levels in blood rabbits have increased, as parathyroid hormonal emissions increase and reduction calcium in the blood. In the diet of eggshells only, calcium in the blood of the rabbits increases, therefore, it is possible that the parathyroid hormones have increased and the calcium levels in the blood have decreased.

As showed in Figure 6, the amount of calcium released in manure of rabbits, fed with eggshells, was 3 times higher at the 14th day. On the other hand, rabbit fed with test sample was 20% higher than control, but 2.5 times less than the rabbits fed with eggshells. According to the results of the study, the test sample is better absorbed than the eggshell.

## Conclusion

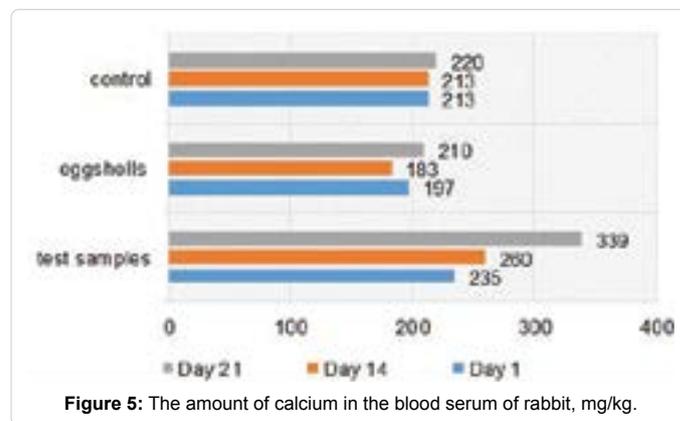


Figure 5: The amount of calcium in the blood serum of rabbit, mg/kg.

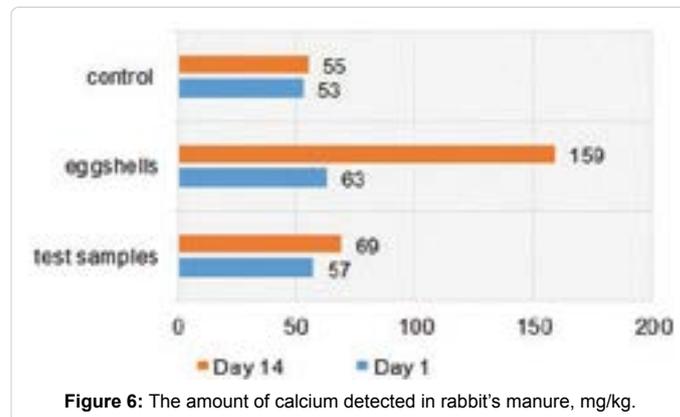


Figure 6: The amount of calcium detected in rabbit's manure, mg/kg.

- Dissolved eggshell in citric acid and pure lemon juice preparing extracts and drying for 5.0-5.5 hours at 50°C is determined as the most appropriate condition.
  - On average, amount of ash in our samples 43.1% less than the ash control sample, however the calcium content of the extracts is similar. In order to the result, the eggshell is extracted with 8% citric acid, which has the highest level of calcium content.
  - The amount of calcium contained in blood serum of the rabbit fed with test samples showed that 35.2% more than the control rabbit at 21 day, and the amount of calcium released in manure were 2.5 times less than rabbits fed with the eggshell only, indicating that calcium absorption is improving.
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