The Journal of Applied Science
วารสารวิทยาศาสตร์ประยุกต์
ISSN 1513-7805 Printed in Thailand

Vol. 16 No. 2: 1-7 [2017]
doi: 10.14416/j.appsci.2017.09.001

Research Article

Calcium chloride produced from eggshell for vegetable washing

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Received: 4/07/2017; Accepted: 26/09/2017

Abstract

The extraction of calcium chloride ($CaCl_2$) from an eggshell waste and its application were investigated. $CaCl_2$ was extracted using 4, 6 and 10% (w/v) hydrochloric acid (HCl) solutions and a drying time was optimized. The optimum condition for $CaCl_2$ extraction was a 10% (w/v) HCl solution and 3 hours of a drying, which gave the highest yield of 16.07 g $CaCl_2$/ 20 g eggshell waste containing with 86.52% (w/w) calcium chloride anhydrous. For the inhibition of pathogens by calcium chloride, both *Escherichia coli* and *Staphylococcus aureus* were inhibited by 80 g/l of $CaCl_2$ extracting with 10% (w/v) HCl solution greater than that with 6% and 4% (w/v) HCl solutions. In addition, $CaCl_2$ was effective to inhibit *E. coli* (67.48%) more than *S. aureus* (40.95%). After 10 minute-immersion in a $CaCl_2$ extracting with 10% (w/v) HCl solution, washing with 1% $CaCl_2$ solution for cucumber (*Cucumis sativus* Linn.) and 2% $CaCl_2$ solution for cabbage (*Brassica oleracea* L. var. capitata) were enough to remove cadmium. Besides, the highest cadmium removal efficiency in a sweet basil leaf (*Ocimum basillicum* Linn.) was found in a 3% (w/v) $CaCl_2$ solution with 20 minutes of immersion. In a firmness test, immersion of cabbage and cucumber for 10 minutes in a 2% $CaCl_2$ solution was enough to increase a firmness value. The firmness values of cabbage and cucumber were 346.17 and 1,049.63 g, respectively. For a firmness test of sweet basil leaf, mostly there was no significant difference between immersion in the $CaCl_2$ solutions and in distilled water. The results, overall, suggested that $CaCl_2$ extracting from the eggshell could be effectively used for inhibition of *E. coli* and *S. aureus*, cadmium removal, and enhancing firmness and also used as vegetable cleaning

Keywords: cadmium removal, calcium chloride, eggshell waste, firmness

Introduction

The chicken egg supplies from the egg farms and egg industries in Thailand are approximately 12,000 million eggs per year (Office of agricultural economics, 2016). A great amount of eggs was consumed in the food-processing industries and the households leading to a large amount of eggshell waste. This becomes a major problem because of pollution and the costs for transportation to landfills, disposal area, and elimination of eggshell waste. In order to minimize disposal and waste management costs, Domrongpokkaphan (2012) reported the conversion of eggshell waste into valuable products, particularly calcium chloride ($CaCl_2$). In the

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literatures, $CaCl_2$ had been applied to increase firmness in fruits (Vongsawasdi et al., 2009). For an environmental management, Makino et al. (2007) had applied $CaCl_2$ to remove cadmium contaminated in paddy soils. However, the application of $CaCl_2$ to inhibit the pathogens has not been reported. Thus, this study aimed to optimize the hydrochloric acid concentration for the extraction of $CaCl_2$ from an eggshell waste, investigate the potential of $CaCl_2$ produced from eggshell waste to inhibit the pathogens, remove cadmium and enhance a firmness of vegetables.

Materials and methods

Effect of hydrochloric acid concentration on the extraction of an eggshell waste into $CaCl_2$ form was examined. Eggshell waste was obtained from canteen of King mongkut's university of technology north bangkok. After membrane removing and washing, an eggshell was pulverized using a mortar into a powder. After that, 20 g of eggshell powder was extracted with 4, 6 and 10% (w/v) HCl concentrations. A ratio of an eggshell waste powder to HCl solution was 1: 15 (w/v). The supernatant from an extraction process was heated at temperature of 200 °C until dried (modified from Garnjanagoonchorn & Changpuak, 2007). An amount of calcium chloride anhydrous was analyzed using Complexometric titration method (Harris, 2003).

The solubility of $CaCl_2$ powder extracting from an eggshell waste was studied. A 0.2 g of $CaCl_2$ powder extracting from eggshell waste was dissolved in 1 ml of distilled water and mixed until homogeneous. This step was repeated until non-homogeneous was observed.

To investigate antipathogen potential of $CaCl_2$ extracting from eggshell waste by broth challenge assay, *Escherichia coli* and *Staphylococcus aureus* were chosen for the representative strains of gram-negative and gram-positive bacteria, respectively. 80 g/l of $CaCl_2$ extracting with 4, 6 and 10% HCl solutions was investigated for the inhibition of pathogens.

Each $CaCl_2$ solution was added into a tube, which was independently contained with *E. coli* and *S. aureus* of 10^3 colony forming units (cfu) per ml. After incubation for 10 min, 0.1 ml aliquots were spread on plates of nutrient agar (in standard amounts) and the plate was incubated at 37 °C for overnight. After that, a viable colony of *E. coli* and *S. aureus* was quantified. A normal saline and calcium chloride anhydrous were used as a negative and positive control, respectively.

Cadmium removal efficiency of $CaCl_2$ powder extracting from an eggshell waste was evaluated. Raw vegetables such as sweet basil leaf (*Ocimum basilicum* Linn.), cabbage (*Brassica oleracea* var. capitata) and cucumber (*Cucummis sativus* Linn.) were used to study cadmium removal efficiency of $CaCl_2$ extracting with 10% (w/v) HCl solution. These vegetables were soaked in Cd solution at a concentration of 2 mg/ml for 1 hour and then were dried at room temperature. After drying, these vegetables were tested for the removal efficiency with $CaCl_2$ solutions of 1, 2 and 3% (w/v) and immersion time of 10 and 20 minutes. Distilled water was used as a control. An amount of Cd discharged from the vegetables was analyzed using atomic absorption spectrometer 240 Fast Sequential AA (Agilent Technologies, USA).

Effect of calcium chloride on firmness of vegetables was studied. Sweet basil leaf, cabbage and cucumber were immersed in $CaCl_2$ solutions of 1, 2 and 3% (w/v) and immersion time of 10 and 20 minutes. Control samples were immersed in distilled water. Each experiment was performed in 30 samples. After drying for 10 minutes, the firmness values of vegetables were analyzed using Texture analyzer (TAXT2i, England).

Results and discussion

Calcium chloride was extracted from an eggshell (Figure 1) using 4, 6 and 10% (w/v) hydrochloric acid solutions. The results showed that an extraction with 10% HCl solution gave

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the highest yields of $CaCl_2$ powder (80.33%, w/w) with the highest percentage of calcium chloride anhydrous, and the shortest drying time of 3 hours when compared with 6% and 4% HCl solutions as shown in Table 1. It was revealed that high concentration of HCl had a potential to extract $CaCl_2$ from eggshell waste.

Figure 1. $CaCl_2$ from an eggshell waste

Table 1. Various HCl concentrations on an extraction of calcium chloride

HCl concentration (%: w/v)	Drying time (hour)	Yield of calcium chloride (g/ 20 g eggshell waste)	Calcium chloride anhydrous (%: w/w)
4	5	10.24	79.60
6	4	13.61	82.02
10	3	16.07	86.52

$CaCl_2$ powder extracting from various HCl concentrations was tested for its solubility. As a result shown in Table 2, when the concentration of HCl increased, the amount of $CaCl_2$ powder dissolving in water obviously increased. That is, 1.0 g $CaCl_2$ powder, which was the highest amount, extracting with 10% (w/v) HCl solution could dissolve in 1-ml distilled water. It is generally known that $CaCl_2$ is highly soluble in water. Therefore, the solubility increases in corresponding with a high percentage of calcium chloride anhydrous.

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doi: 10.14416/j.appsci.2017.09.001

Table 2. Solubility of $CaCl_2$ powder extracting from eggshell

HCl concentration	An amount of $CaCl_2$ dissolving in 1 ml of distilled water (g)						
(%: w/v)	0.2	0.4	0.6	0.8	1.0	1.2	1.4
4%	+	+	+	-	-	-	-
6%	+	+	+	+	-	-	-
10%	+	+	+	+	+	-	-

\+ refers to all $CaCl_2$ powder dissolving in 1 ml of distilled water
\- refers to $CaCl_2$ powder non dissolving in 1 ml of distilled water

The $CaCl_2$ powder was tested for its antimicrobial activity against pathogenic bacteria, i.e. *Escherichia coli* and *Staphyloccus aureus*, by challenging in a normal saline solution. Initial pure strain of *E. coli* of 4.7 $x10^3$ and *S. aureus* of 4.5 $x10^3$ cfu/ml was used as a control and inoculums. From Figure 2, calcium chloride anhydrous (commercial grade) was a powerful antimicrobial agent because of its high-purity. However, $CaCl_2$ powder of 80 g/l extracting with 10% HCl solution showed higher inhibition for both pathogenic bacteria when compared with 6% and 4% HCl solutions. The viable cells of *E. coli* and *S. aureus* were inhibited to 67.48% and 40.95%, respectively (Figure 2 and 3). When $CaCl_2$ powder was dissolved in water, it created hypochlorous acid. This chemical was recognized as a powerful antimicrobial substance because of its potential oxidizing capacity and cytoplasmic membrane was a key target that involved in the bacterial inactivation by hypochlorous acid. However, at low hypochlorous acid levels, microorganisms might be injured rather than inactivated (Venkobachar et al., 1977; Virto et al., 2005).

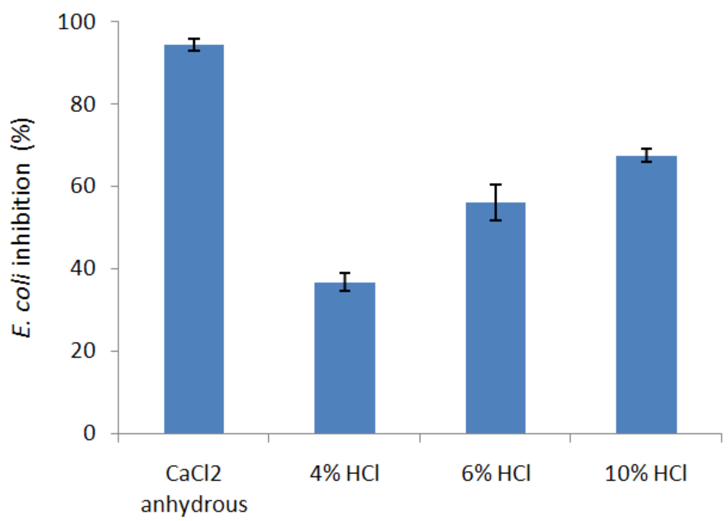

Figure 2. *Escherichia coli* inhibition by $CaCl_2$ extracting from eggshell waste (each value was average±SD)

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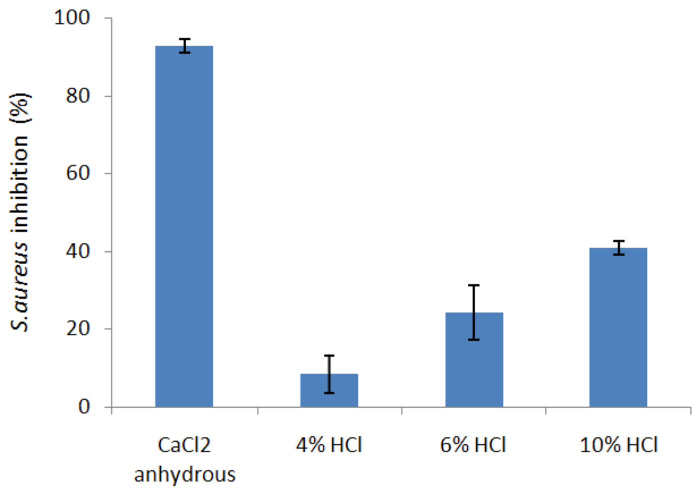

Figure 3. *Staphyloccus aureus* inhibition by $CaCl_2$ extracting from eggshell waste (each value was average±SD)

The efficiency of $CaCl_2$ to remove cadmium from the vegetables was shown in Table 3. It was found that $CaCl_2$ had a potential to discharge cadmium on the surface of the cucumber, cabbage and sweet basil leaf. That is, 1% and 2% (w/v) $CaCl_2$ solutions were enough to remove cadmium in cucumber and cabbage, respectively, at 10 minutes of immersion. However, the highest cadmium removal efficiency in a sweet basil leaf was observed in 3% (w/v) $CaCl_2$ solution and 20 minutes of immersion.

Table 3. The concentration of $CaCl_2$ solution and immersion time on cadmium removal efficiency

Vegetables	Immersion time (minutes)	An average of cadmium concentration (mg/l ±SD)			
		Percentage of $CaCl_2$ solution			Distilled water
		1% (w/v)	2% (w/v)	3% (w/v)	
Sweet basil leaf	10	0.403±0.061[c]	0.406±0.035[c]	0.444±0.028[d]	0.047±0.015[a]
	20	0.369±0.046[b]	0.424±0.014[cd]	0.475±0.034[e]	0.032±0.010[a]
Cabbage	10	0.330±0.039[b]	0.440±0.029[c]	0.451±0.021[c]	0.041±0.001[a]
	20	0.331±0.021[b]	0.445±0.150[c]	0.442±0.034[c]	0.041±0.005[a]
Cucumber	10	0.087±0.003[d]	0.081±0.003[c]	0.078±0.004[b]	0.005±0.001[a]
	20	0.078±0.004[b]	0.082±0.002[c]	0.079±0.001[b]	0.006±0.001[a]

Note: Different letters in the same type of vegetable refers to significant different ($p<0.05$)
All measurements were performed in 8 samples

 doi: 10.14416/j.appsci.2017.09.001

From Table 3, $CaCl_2$ clearly had a potential to discharge cadmium. As described in the experiment of Makino et al. (2007), when $CaCl_2$ was added to soils, Ca ions quickly exchanged with Cd ions on the surface of soil. Therefore, washing with $CaCl_2$ led to a formation of Cd-Cl complex and increasing Cd discharging from soil. In addition, Ok et al. (2011) reported that $CaCl_2$ was able to extract the adsorbed cations because it had the same ionic charge.

For a firmness test, 3 vegetables were immersed in the 1, 2 and 3% (w/v) $CaCl_2$ solutions. Mostly, there was no significant difference of firmness in a sweet basil leaf between the $CaCl_2$ solutions and distilled water. However, a 2% $CaCl_2$ solution for 10 minutes of immersion showed the highest firmness value in cabbage and cucumber.The firmness value of cabbage and cucumber was 346.17±61.15 and 1,049.63±83.91 g, respectively, (Table 4). $CaCl_2$ solution had been used as a firming agent for vegetables including whole and fresh-cut fruits (Izumi & Watada, 1994; Luna-Guzman et al., 1999; Garcia et al., 1996). $CaCl_2$ had a firming effect due to a cross-linking of both cell wall and middle-lamella pectin by calcium ions (Manolopoulou & Varzakas, 2011).

Table 4. Firmness value of sweet basil leaf, cabbage and cucumber after immersion with $CaCl_2$ solutions

Vegetables	Immersion time (minutes)	An average of firmness value (g±SD)			
		Percentage of $CaCl_2$ solution			Distilled water
		1% (w/v)	2% (w/v)	3% (w/v)	
Sweet basil leaf	10	247.03±79.13[a]	273.21±83.40[b]	259.79±79.78[a]	223.49±76.04[a]
	20	252.49±66.45[a]	265.42±76.65[a]	258.79±61.22[a]	231.50±81.14[a]
Cabbage	10	206.59±41.92[a]	346.17±61.15[b]	343.24±63.42[b]	166.77±28.18[a]
	20	216.26±63.67[a]	370.24±75.56[b]	347.27±74.05[b]	177.13±37.35[a]
Cucumber	10	1,010.59±93.53[a]	1,049.63±83.91[b]	1,030.24±83.39[b]	968.80±94.21[a]
	20	1,009.74±85.67[a]	1,042.97±92.21[b]	1,037.97±66.30[b]	964.36±67.31[a]

Note: Different letters in the same type of vegetable refers to significant different ($p<0.05$)
All measurements were performed in 30 samples

Conclusion

The highest yield calcium chloride of 80.35% (w/w) was obtained from the extraction of 10% (w/v) HCl solution. $CaCl_2$ concentration of 80 g/l was found to inhibit *E. coli* greater than *S. aureus*. At the same soaking time for 10 minutes, cucumber required the lowest percentage of $CaCl_2$ solution to remove cadmium, which better than cabbage and sweet basil leaf. In addition, the 2% $CaCl_2$ solution with 10 minutes of immersion showed the highest firmness value in the cabbage and cucumber. However, mostly there was no significant difference of firmness value in sweet basil leaf between $CaCl_2$ solutions and distilled water.

Acknowledgement

The authors are grateful to the support provided by the Faculty of Applied Science, King Mongkut's University of Technology North Bangkok (Thailand) under grant No. 5544104.

References

Domrongpokkaphan, V. (2012). Eggshell utilization technology. *Journal of Applied Science*, *11*:75-83. (in Thai)

Garcia, J. M., Herrera, S. & Morilla, A. (1996). Effects of postharvest dips in calcium chloride on strawberry. *Journal of Agricultural and Food Chemistry*, *44*:30-33.

Garnjanagoonchorn, W. & Changpuak, A. (2007). Preparation and partial characterization of eggshell calcium chloride. *International Journal of Food Properties*, *10*(3): 497-503. http://dx.doi.org/10.1080/10942910600919484

Harris, D. C. (2003). *Quantitative Chemical Analysis* (6th ed.). New York: W.H. Freeman and Company.

Izumi, H. & Watada, A. E. (1994). Calcium treatments affect storage quality of shredded carrots. *Journal of Food Science*, *59*:106-109. doi: 10.1111/j.1365-2621.1994.tb06908.x

Luna-Guzman, I., Cantwel, M. & Barrett, D. M. (1999). Fresh-cut cantaloupe: effects of $CaCl_2$ dips and heat treatments on firmness and metabolic activity. *Postharvest Biology and Technology*, *17*:201-213. https://doi.org/10.1016/S0925-5214(99)00048-4

Makino, T., Kamiya, T., Takano, H., Itou, T., Sekiya, N., Sasaki, K. et al. (2007). Remediation of cadmium-contaminated paddy soils by washing with calcium chloride: verification of on-site washing. *Environmental Pollution*, *147*:112-119. https://doi.org/10.1016/j.envpol.2006.08.037

Manolopoulou, E. & Varzakas, T. (2011). Effect of storage conditions on the sensory quality, colour and texture of fresh-cut minimally processed cabbage with the addition of ascorbic acid, citric acid and calcium chloride. *Food and Nutrition Sciences*, *2*:956-963. doi: 10.4236/fns.2011.29130

Office of agricultural economics. (2016). *Agricultural statistics of Thailand 2015*. Bangkok, Thailand: Office of National Buddhism Press.

Ok, Y. S., Lee, S. S., Jeon, W. T., Oh, S. E., Usman, A. R. A. & Moon, D. H. (2011). Application of eggshell waste for the immobilization of cadmium and lead in a contaminated soil. *Environmental Geochemistry and Health*, *33*:31–39. doi 10.1007/s10653-010-9362-2

Venkobachar, C., Iyengar, L. & Rao, A. V. S. P. (1977). Mechanism of disinfection: effect of chlorine on cell membrane functions. *Water Research*, *11*:727-729. https://doi.org/10.1016/0043-1354(77)90114-2

Virto, R., Manas, P., Alvarez, I., Condon, S. & Raso, J. (2005). Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. *Applied and Environmental Microbiology*, *71*(9):5022-5028. doi: 10.1128/AEM.71.9.5022-5028.2005

Vongsawasdi, P., Nopharatana, M., Viriyaprakob, P. & Ditnoi, R. (2009). Fresh-Cut Guava: part I effects of calcium chloride and heat treatment on physical and chemical properties of Guava, *Agricultural Science Journal*. *40*(3):125-128. (in Thai)